



US008975229B2

(12) **United States Patent**
Tsujikawa et al.

(10) **Patent No.:** **US 8,975,229 B2**
(45) **Date of Patent:** **Mar. 10, 2015**

(54) **METHODS FOR TREATING A DISEASE
CAUSED BY CHOROIDAL
NEOVASCULARIZATION**

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(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

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(21) Appl. No.: **13/377,110**

(22) PCT Filed: **Jun. 10, 2010**

(86) PCT No.: **PCT/JP2010/003871**

§ 371 (c)(1),
(2), (4) Date: **Mar. 8, 2012**

(87) PCT Pub. No.: **WO2010/143435**

PCT Pub. Date: **Dec. 16, 2010**

(65) **Prior Publication Data**

US 2012/0156233 A1 Jun. 21, 2012

(30) **Foreign Application Priority Data**

Jun. 11, 2009 (JP) 2009-140363

(51) **Int. Cl.**

A61K 38/00 (2006.01)

A61P 35/00 (2006.01)

C07K 14/515 (2006.01)

A61P 9/10 (2006.01)

A61P 27/02 (2006.01)

A61K 38/08 (2006.01)

B60N 2/225 (2006.01)

C07K 14/00 (2006.01)

C07K 14/71 (2006.01)

(52) **U.S. Cl.**

CPC **B60N 2/2252** (2013.01); **C07K 14/00**
(2013.01); **C07K 14/71** (2013.01); **A61K 38/00**
(2013.01)

USPC **514/13.3**; 514/20.8; 514/21.6

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides novel pharmaceutical agents
and methods for treating or preventing diseases caused by
neovascularization in human choroid (neovascular macul-
opathy). The present invention provides pharmaceutical com-
positions and vaccines for treating and/or preventing diseases
caused by neovascularization in human choroid (neovascular
maculopathy), comprising at least one type each of a peptide
comprising an amino acid sequence derived from a VEGFR-1
protein and having an activity of inducing cytotoxic T cells,
and a peptide comprising an amino acid sequence derived
from a VEGFR-2 protein and having an activity of inducing
cytotoxic T cells.

22 Claims, 19 Drawing Sheets

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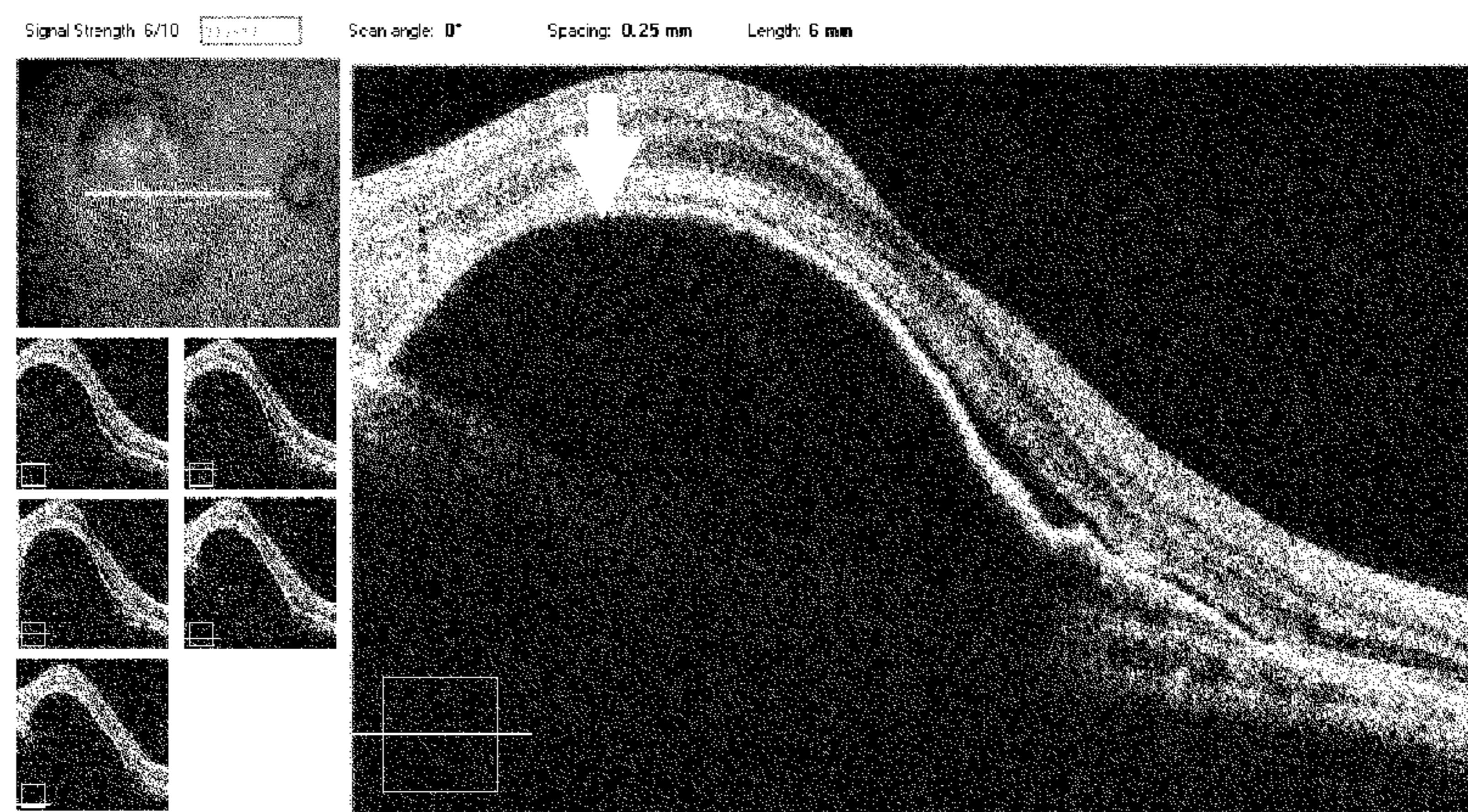
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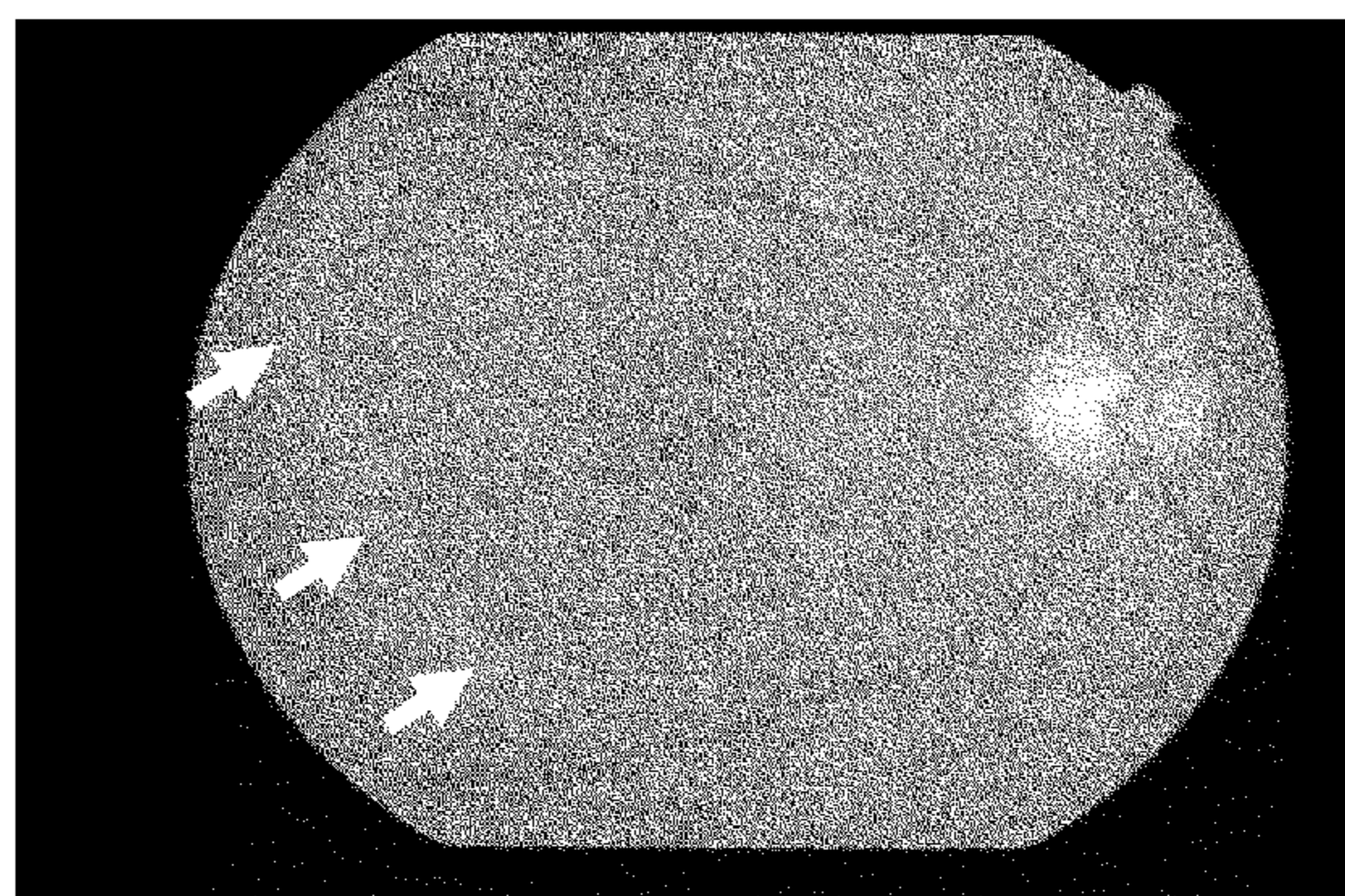
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Fig. 1-1
HLA-A0201-Case1 Pre-treatment

A



B



C

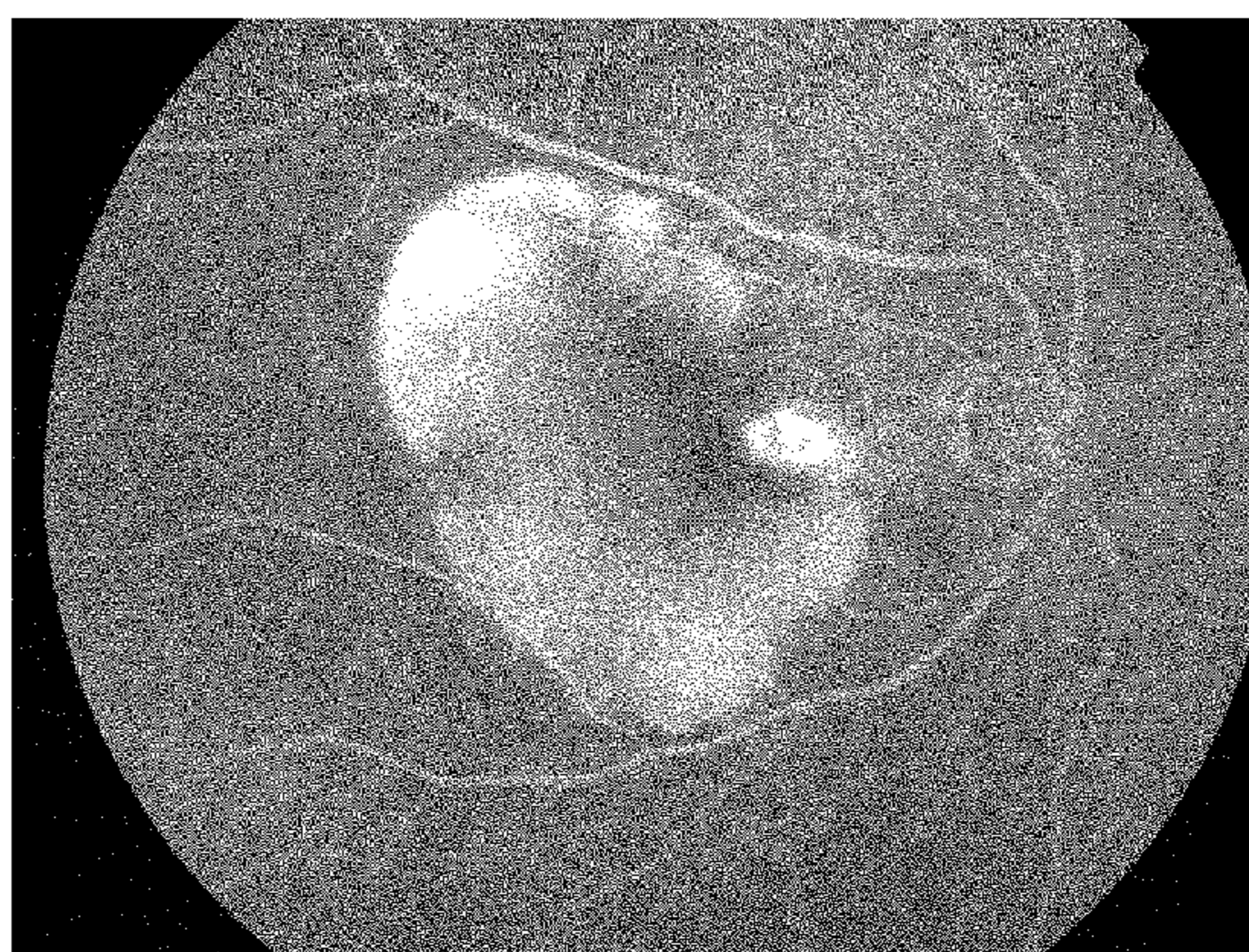
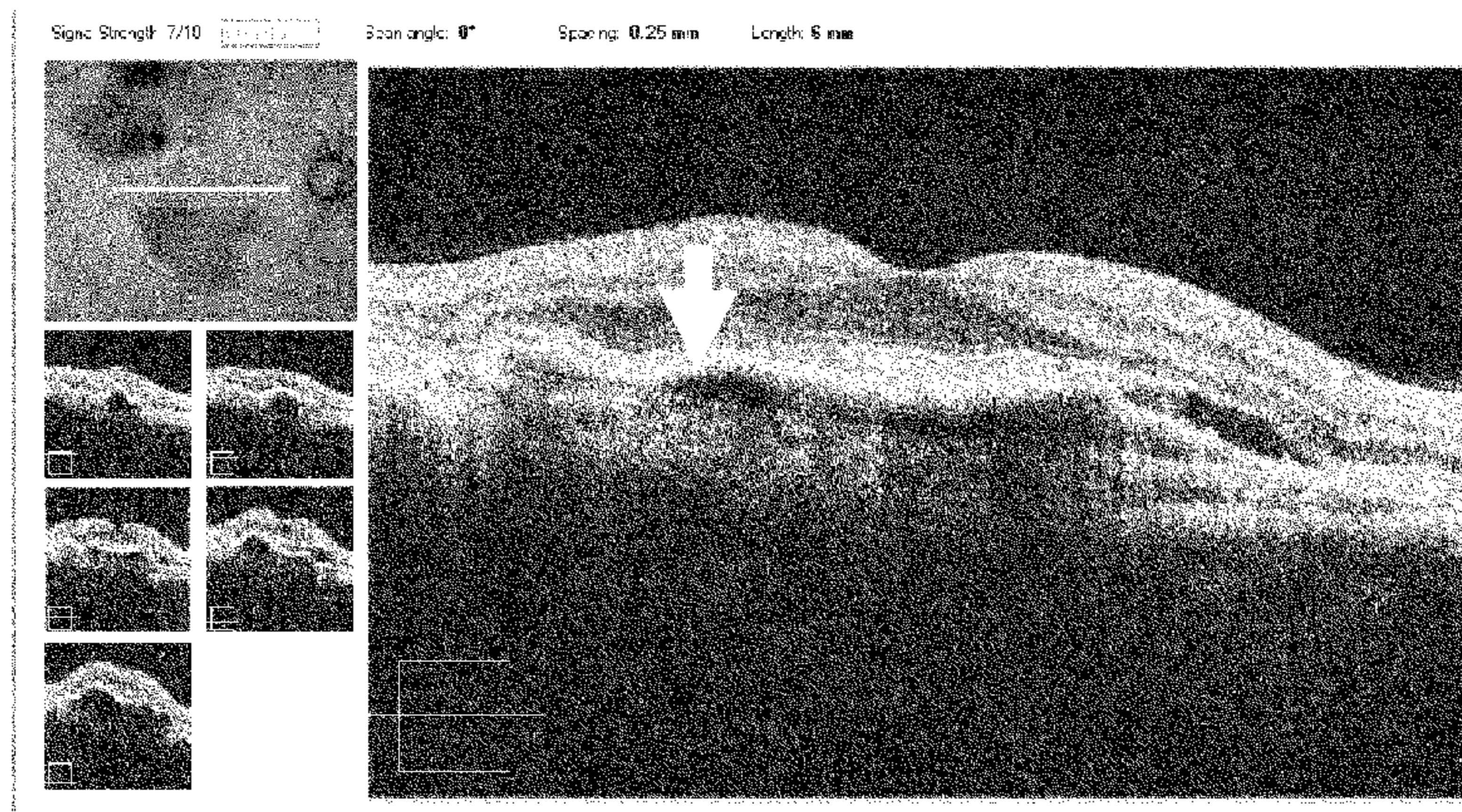
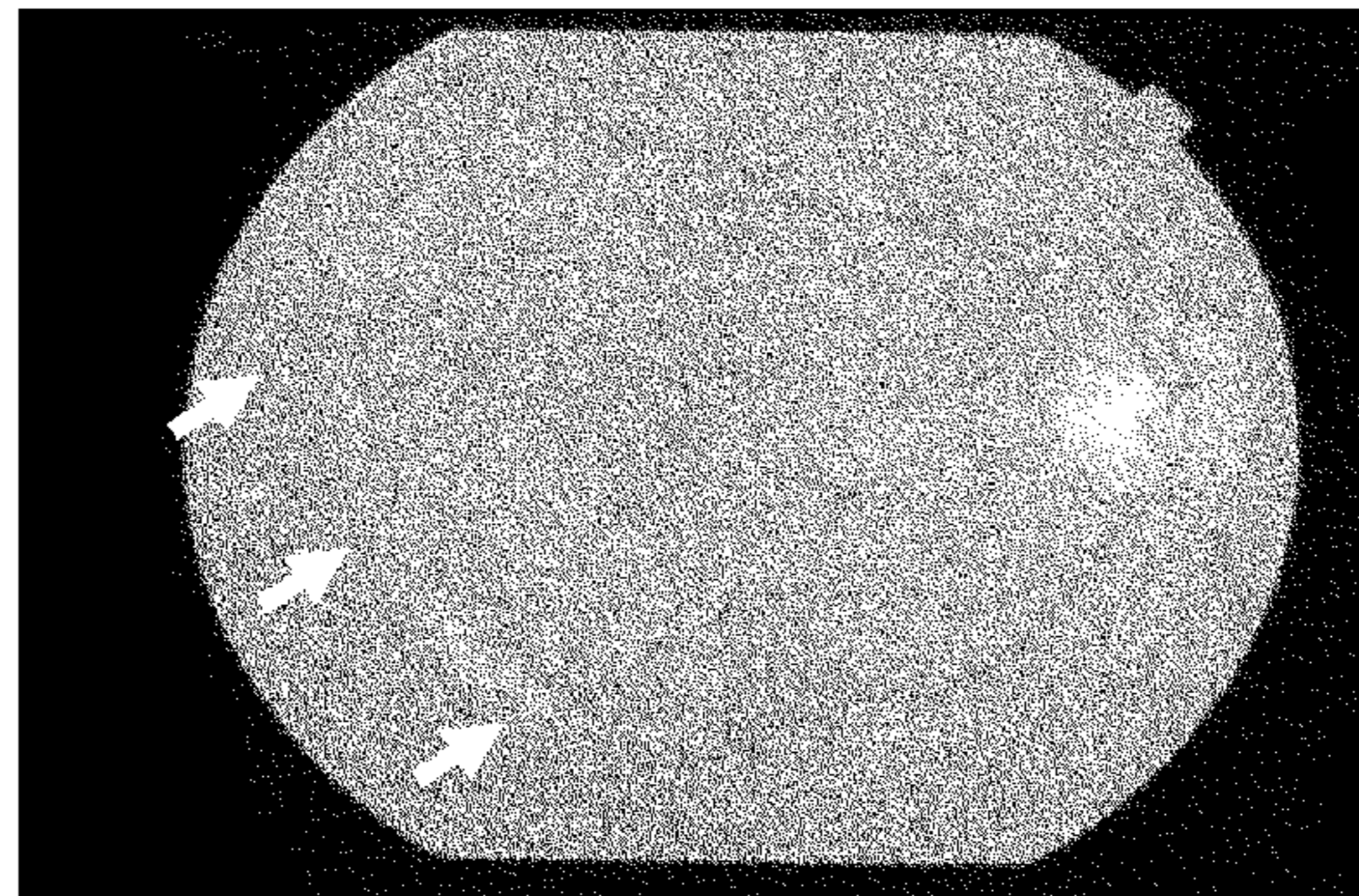


Fig. 1-2
HLA-A0201-Case1 5 months later after starting treatment

D



E



F

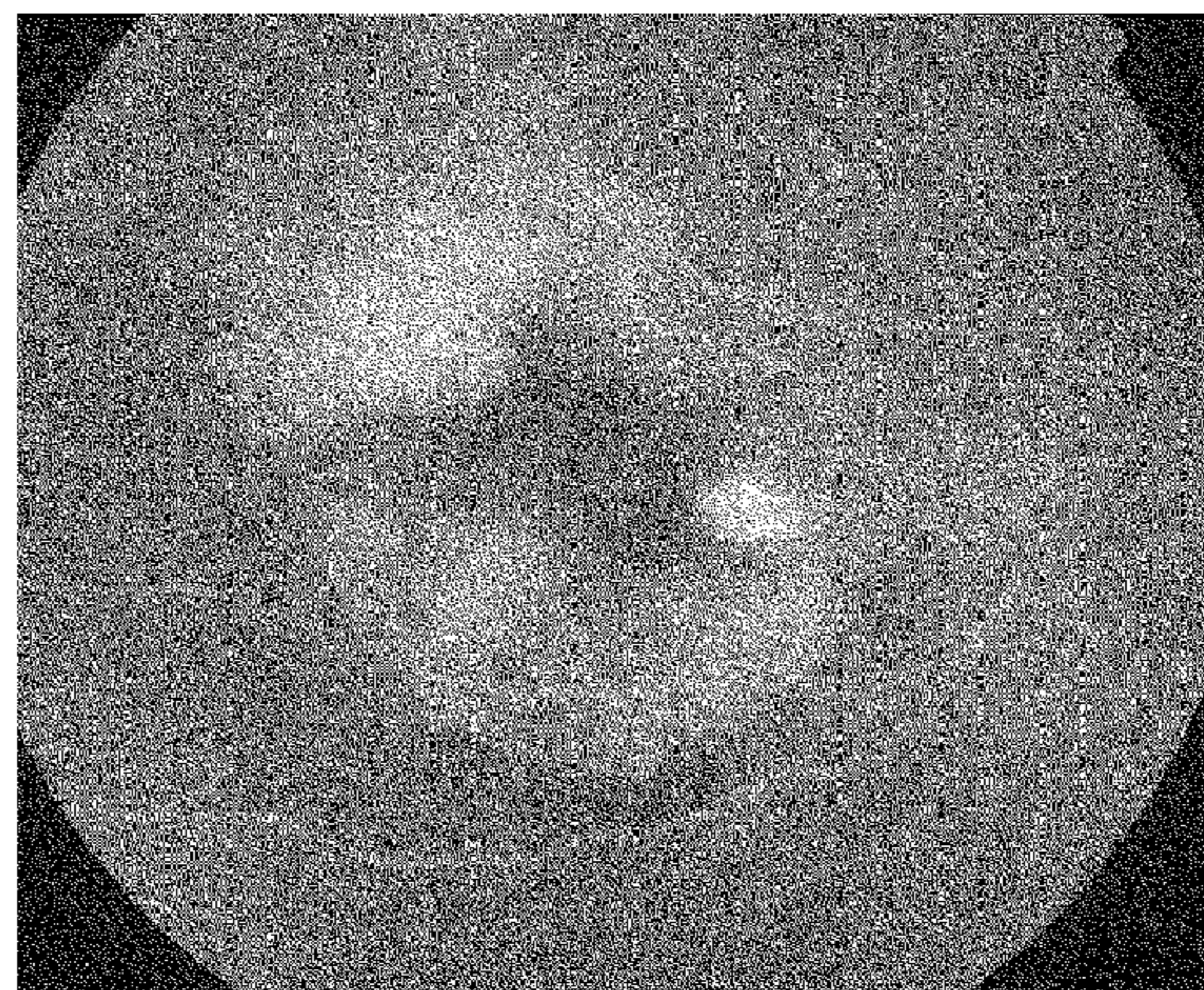
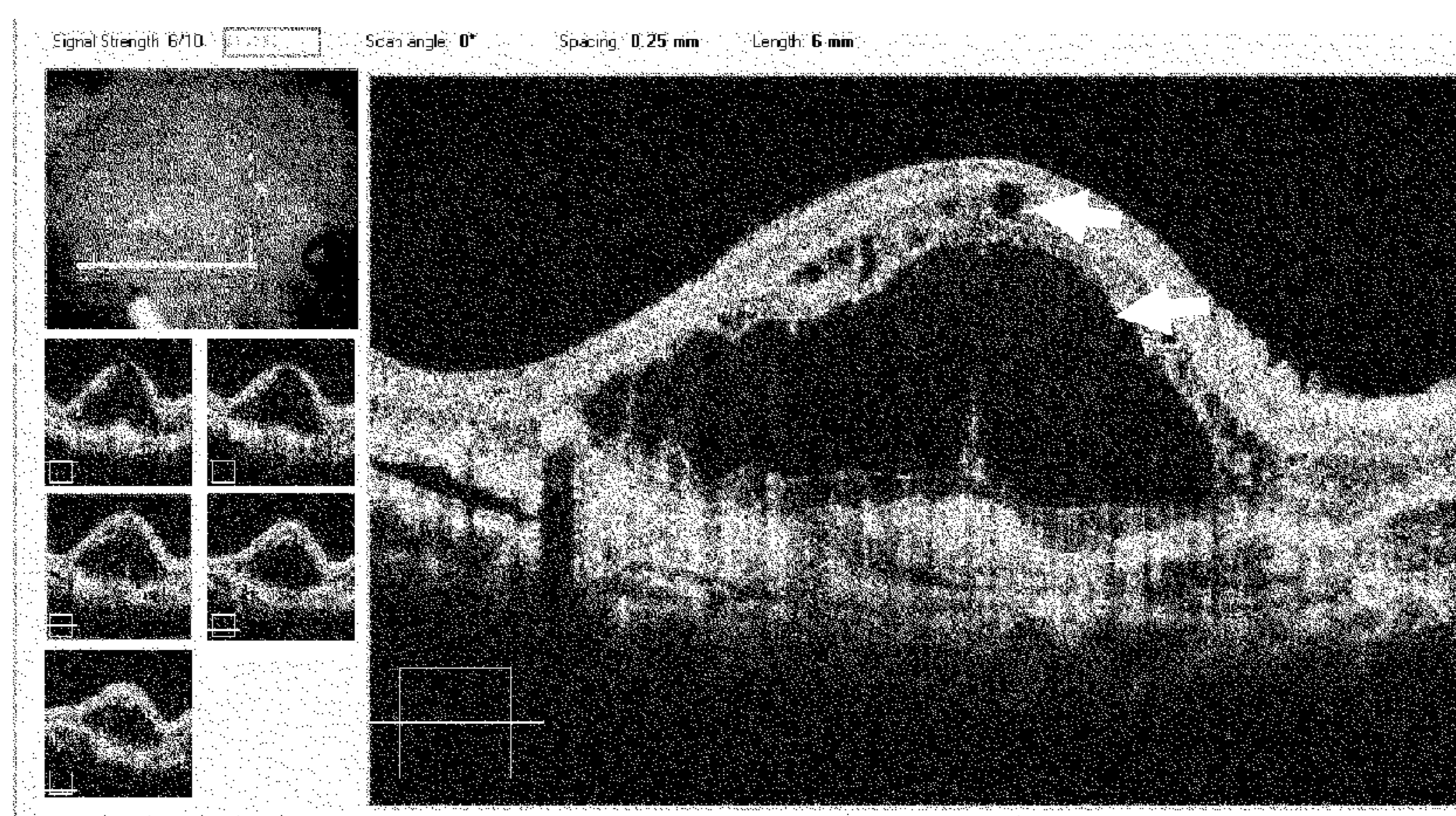


Fig. 2
HLA-A0201-Case3

A Pre-treatment



B One month later after treatment

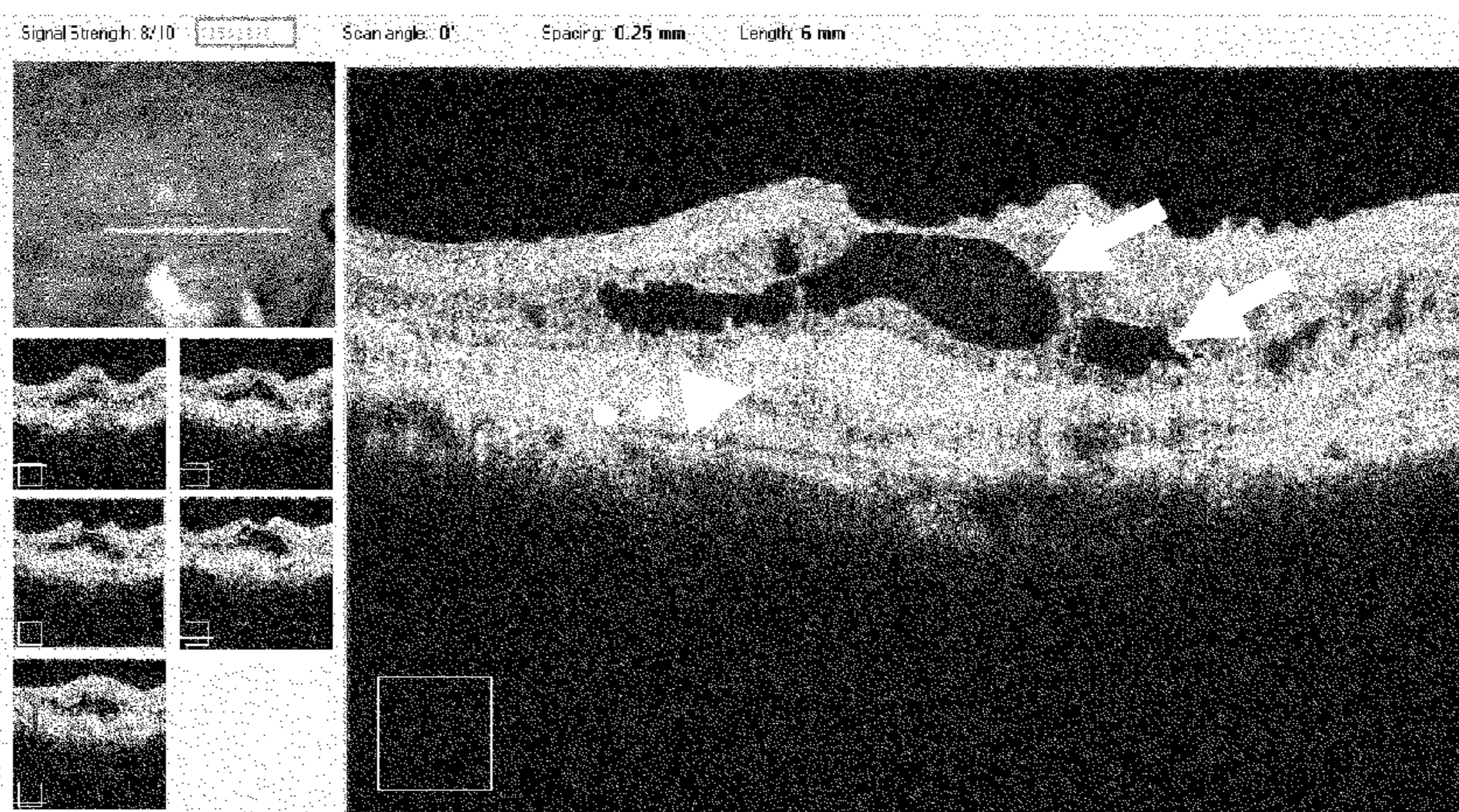


Fig. 3
HLA-A2402-Case1

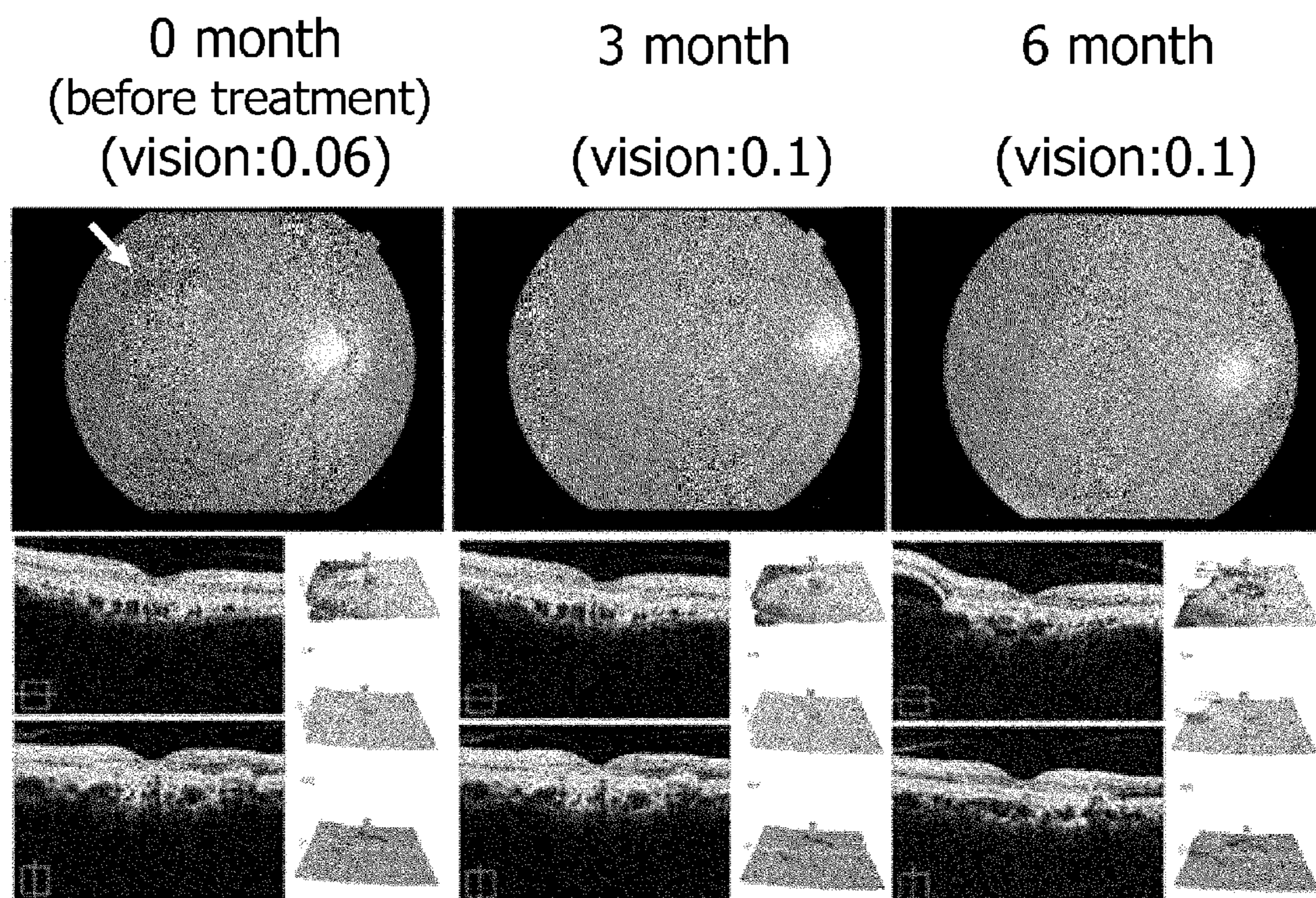
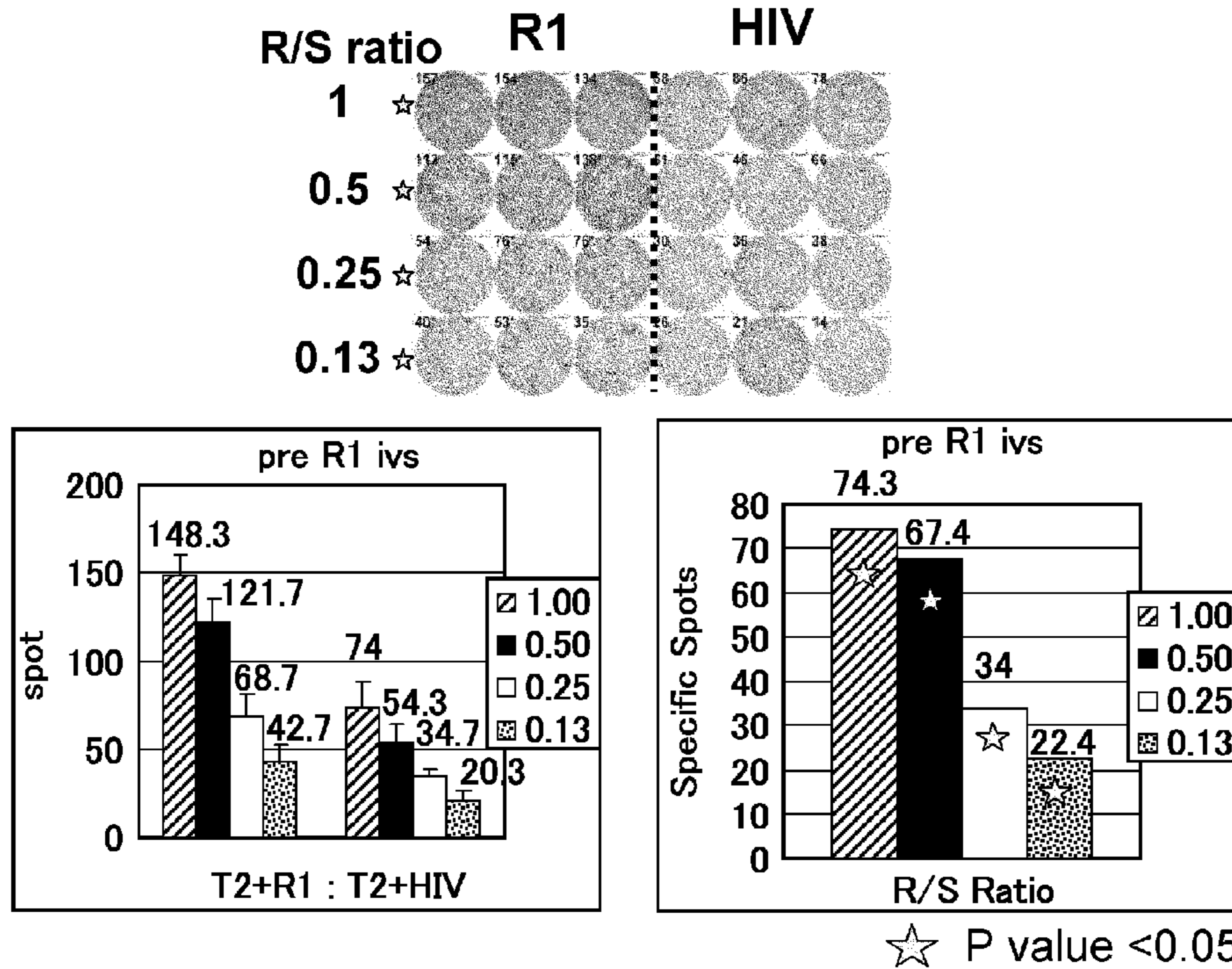


Fig. 4-1

a A0201-Case 1. pre-treatment (VEGFR1)



b A0201-Case 1. post-1course (VEGFR1)

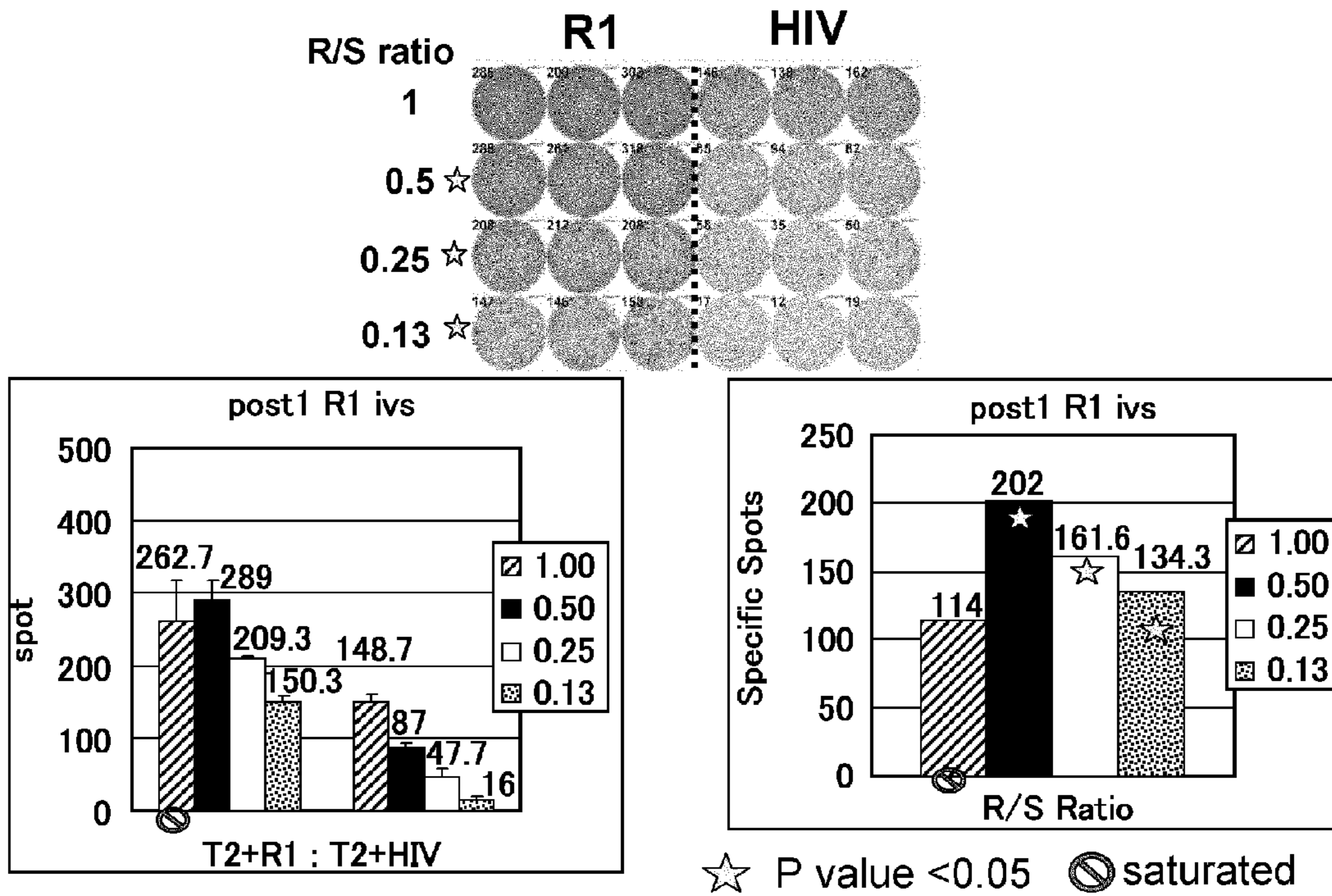
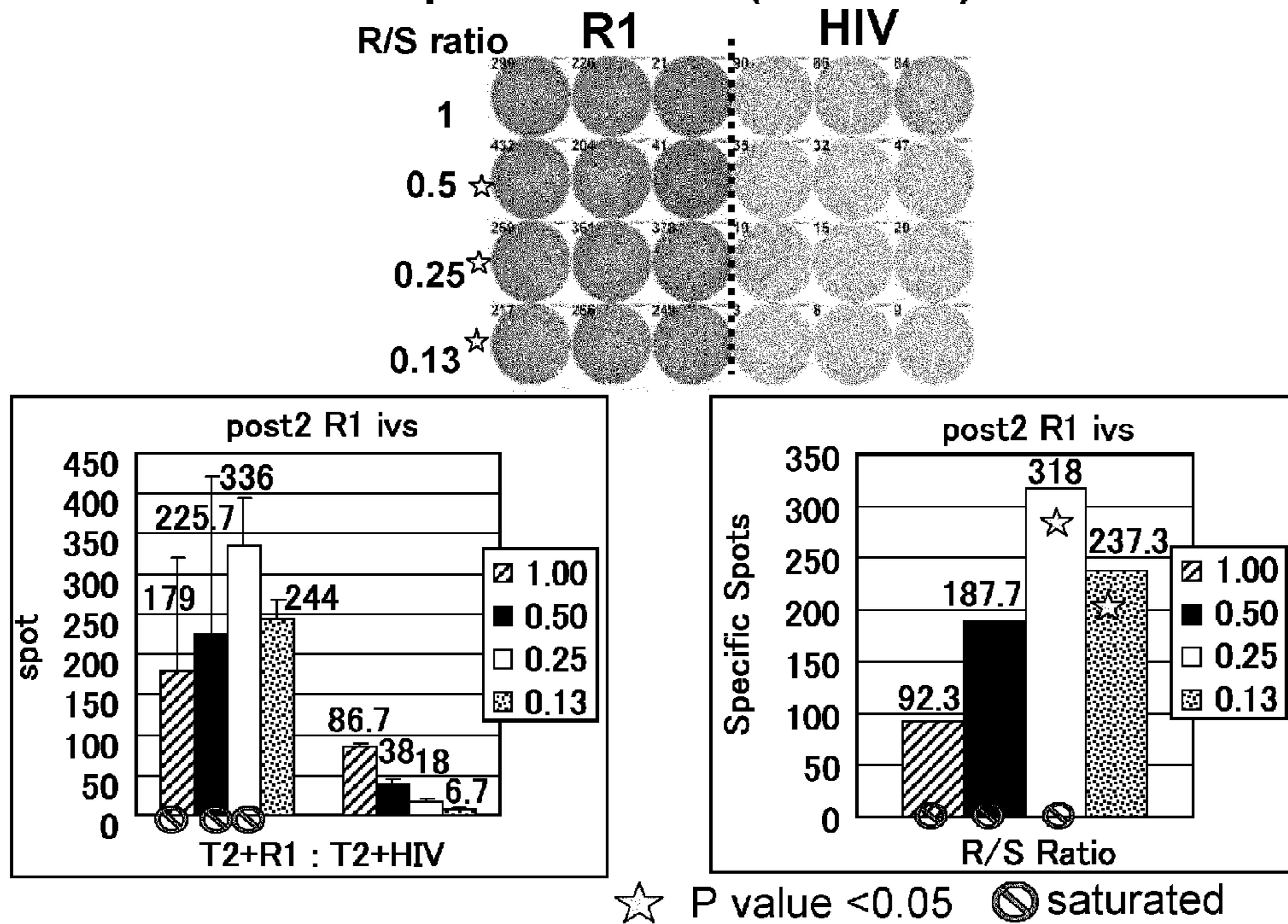


Fig. 4-2

c A0201-Case 1. post-2course (VEGFR1)



d A0201-Case 1. post-3course (VEGFR1)

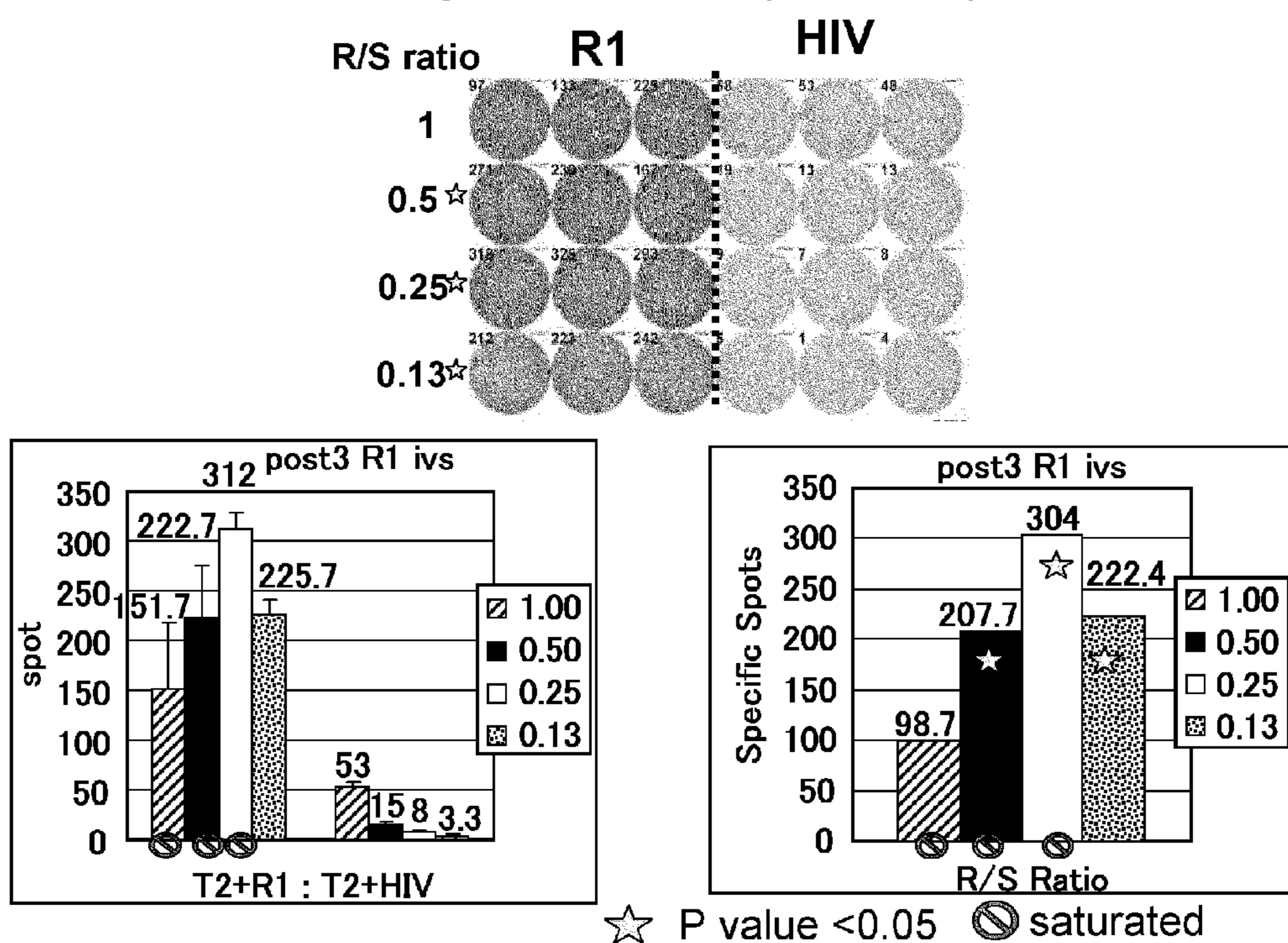
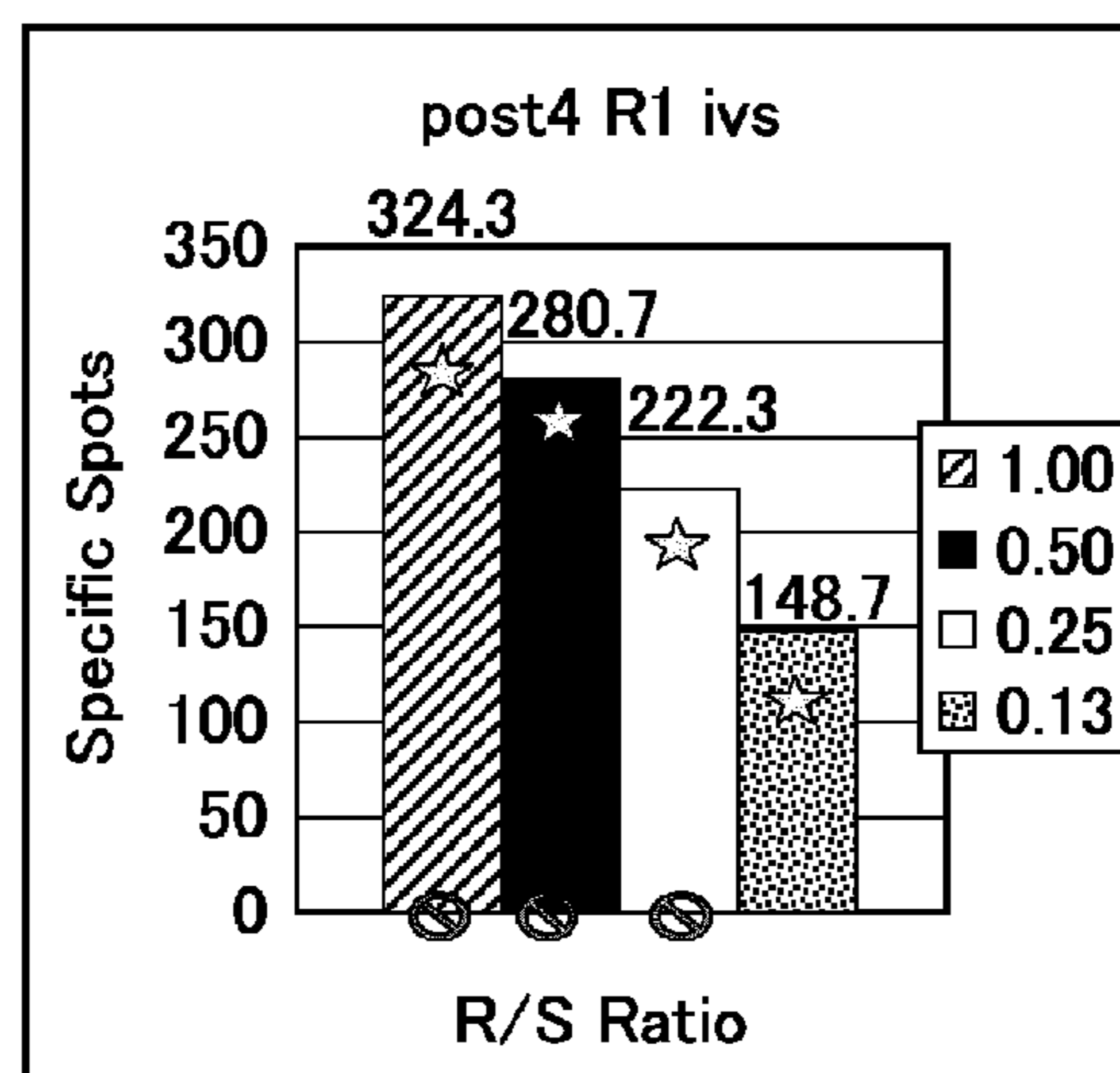
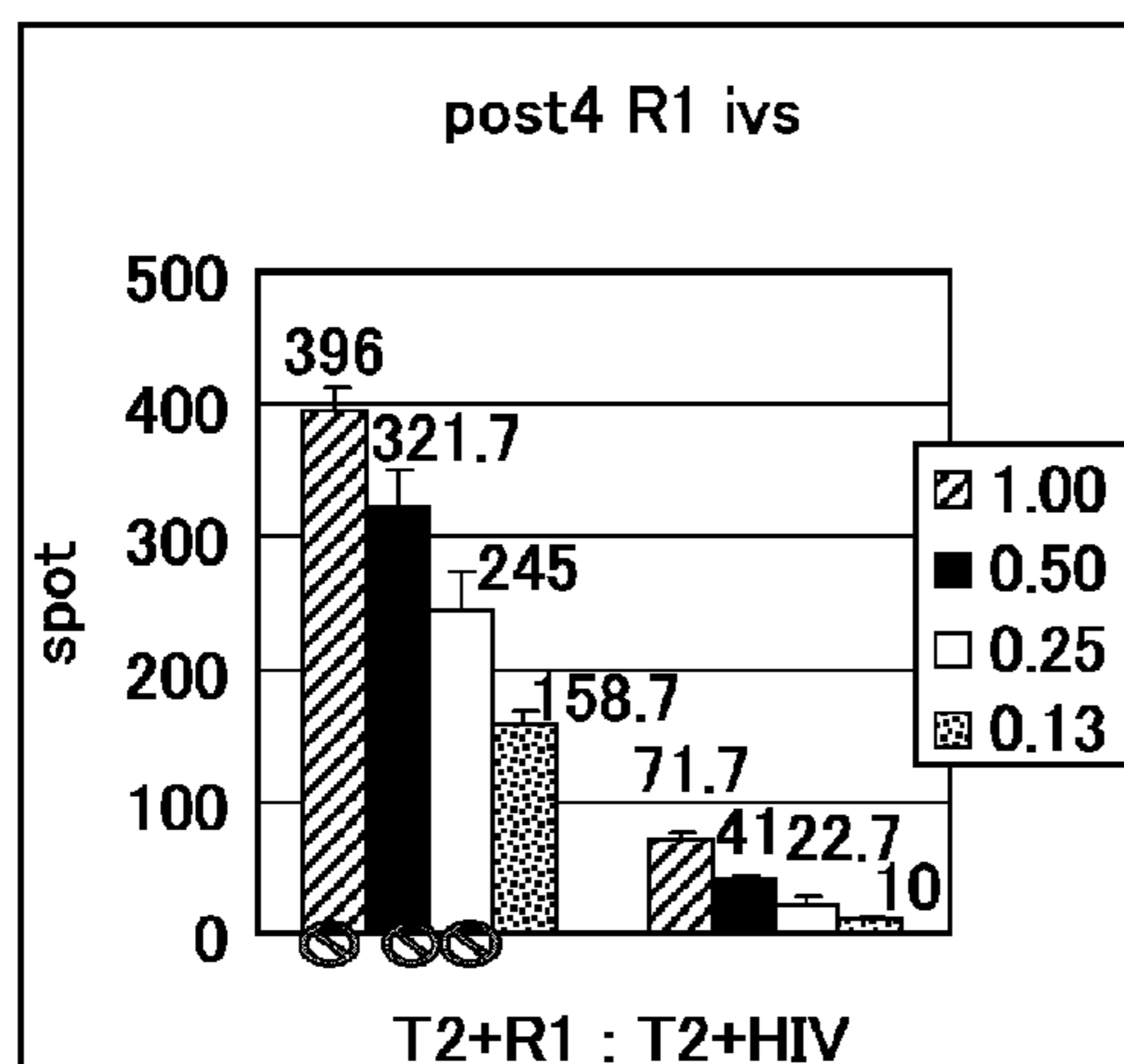
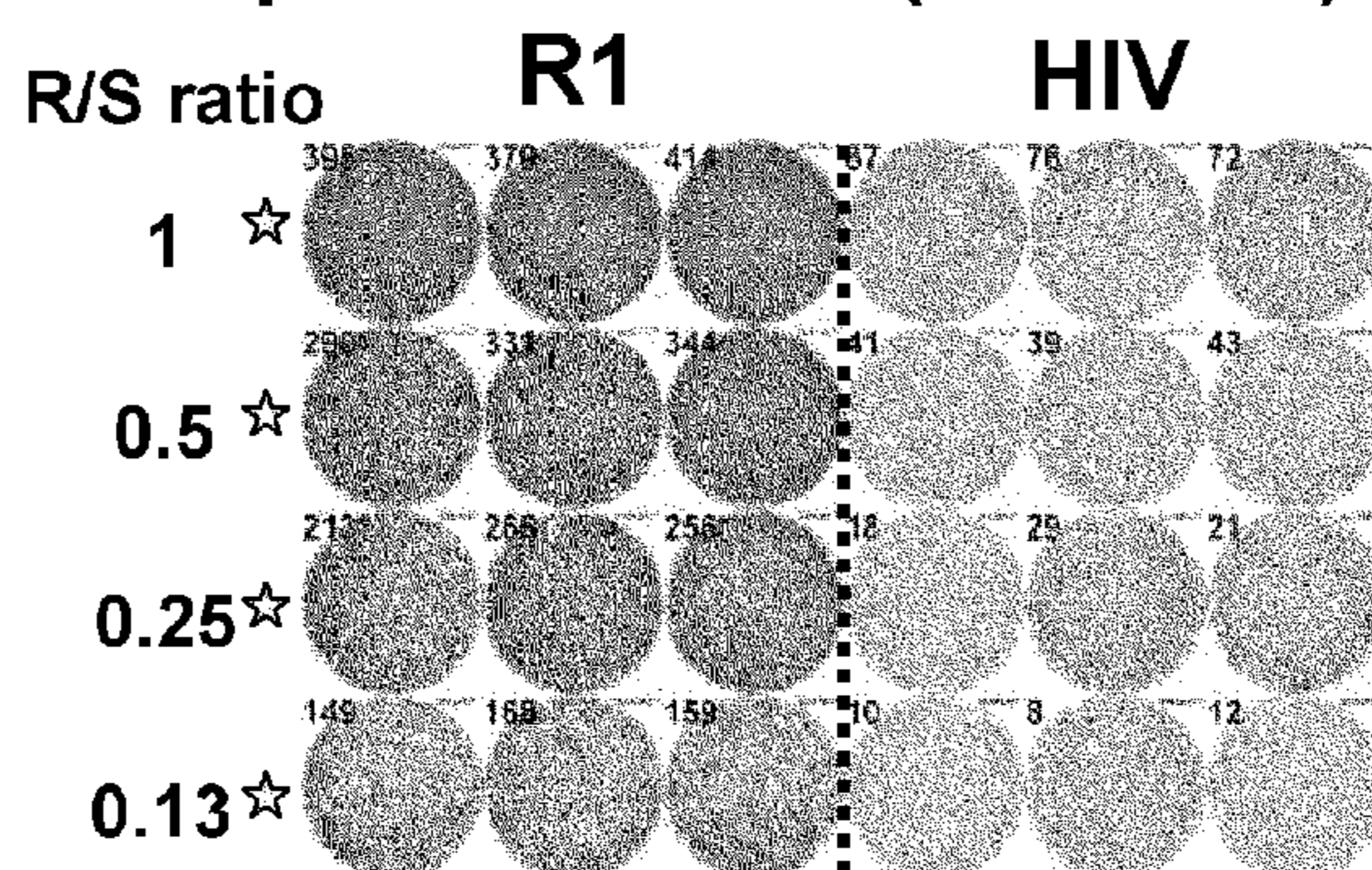


Fig. 4-3

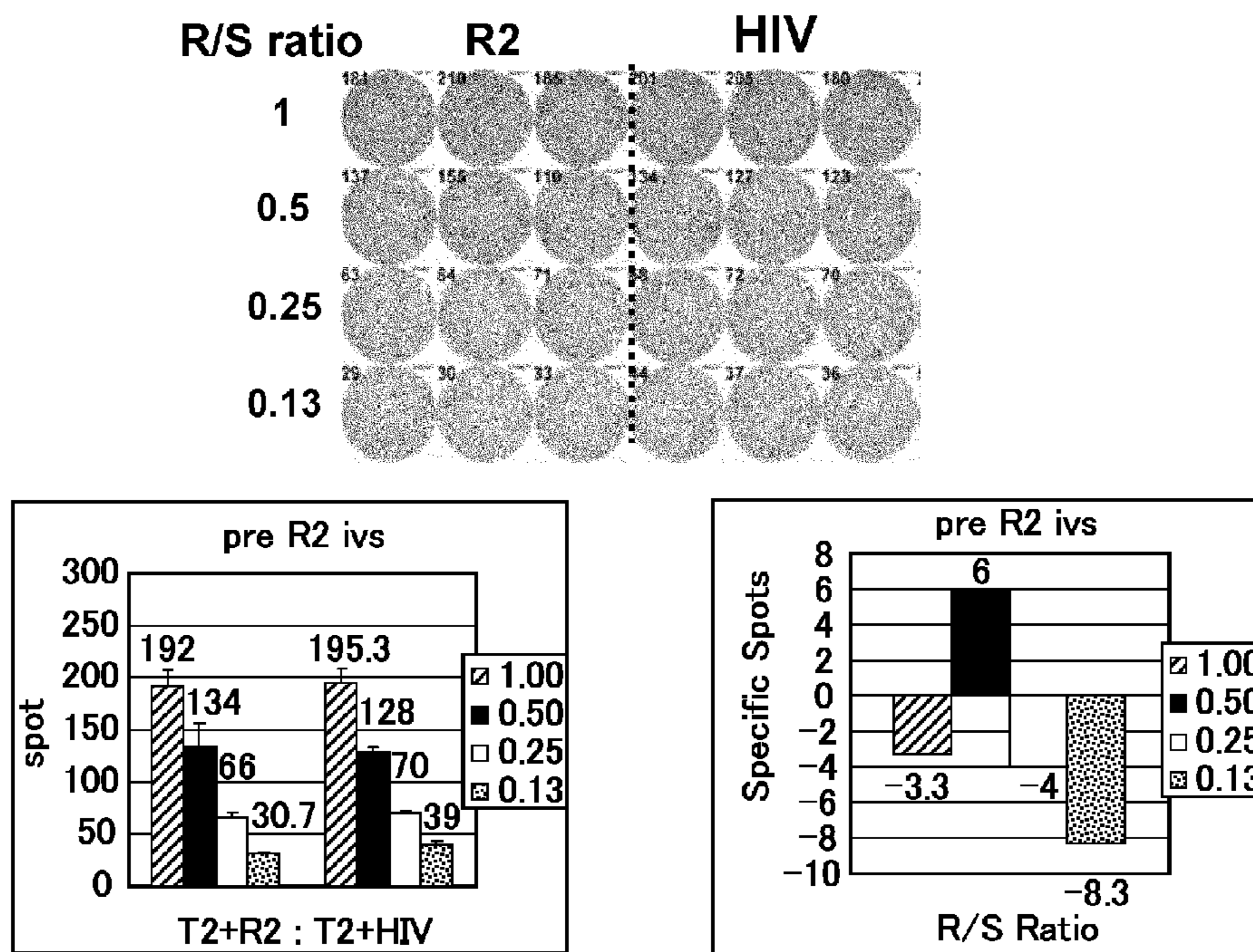
e A0201-Case 1. post-4course (VEGFR1)



☆ P value < 0.05 ⊘ saturated

Fig. 5-1

a A0201-Case 1. pre-treatment (VEGFR2)



b A0201-Case 1. post-1course (VEGFR2)

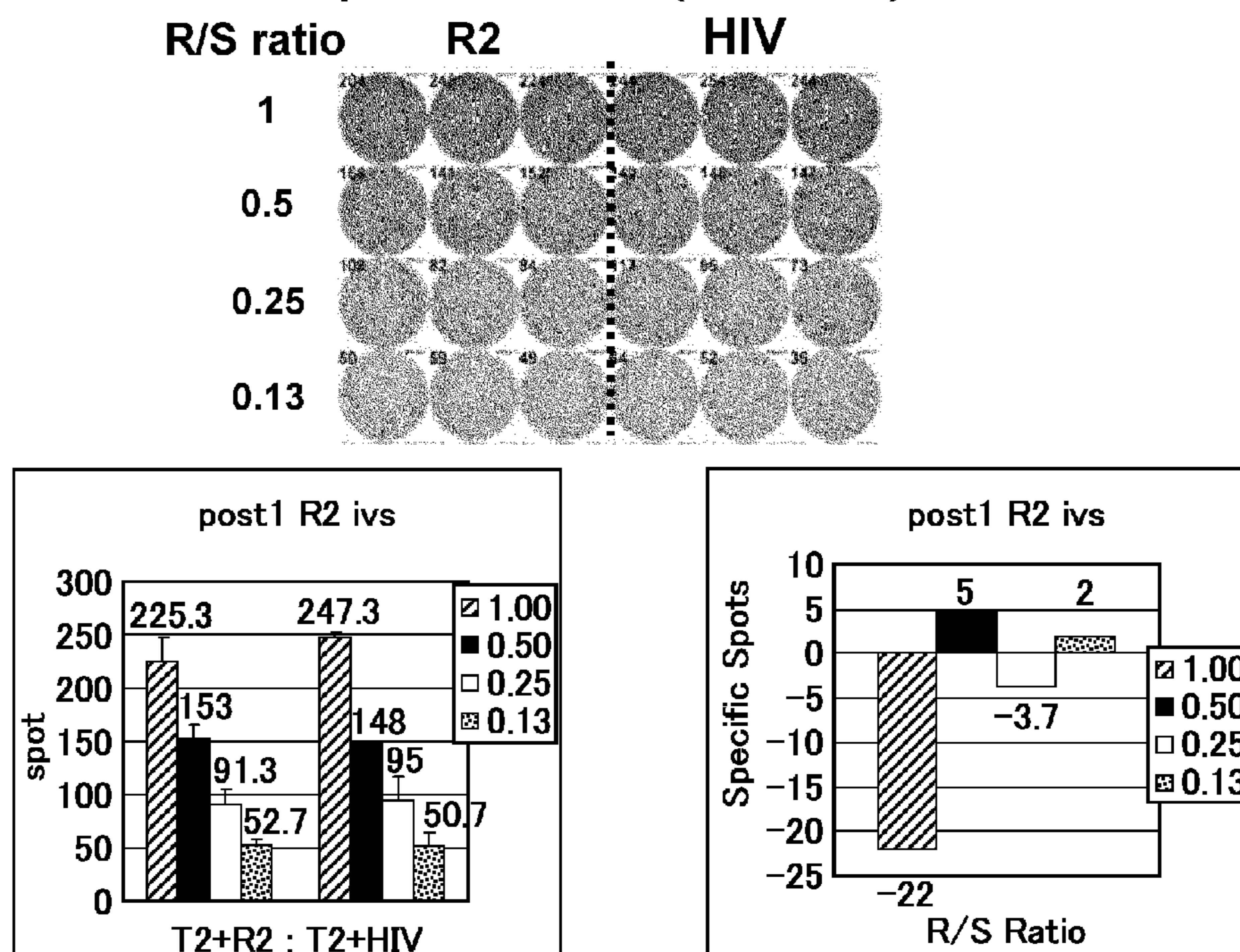
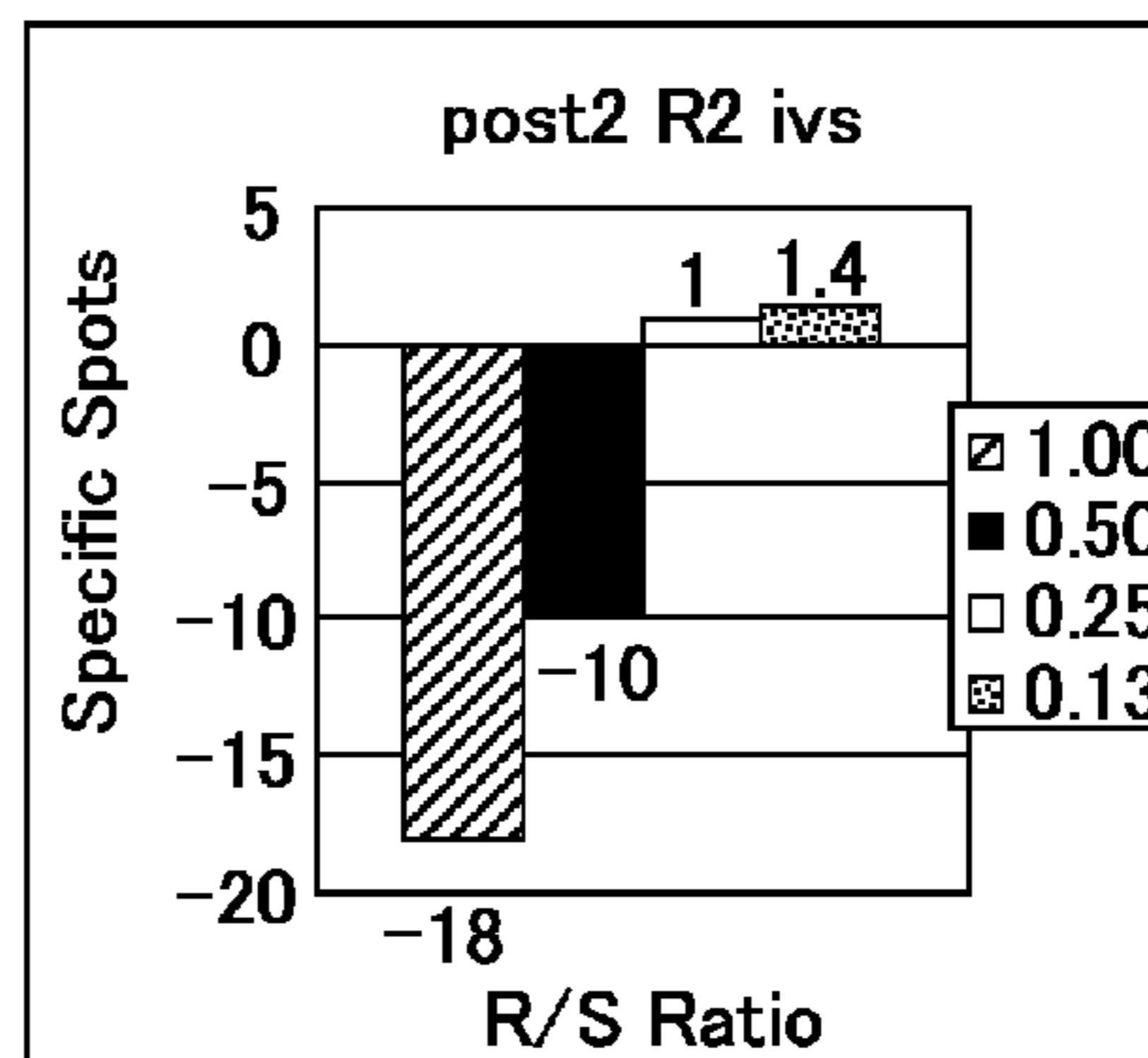
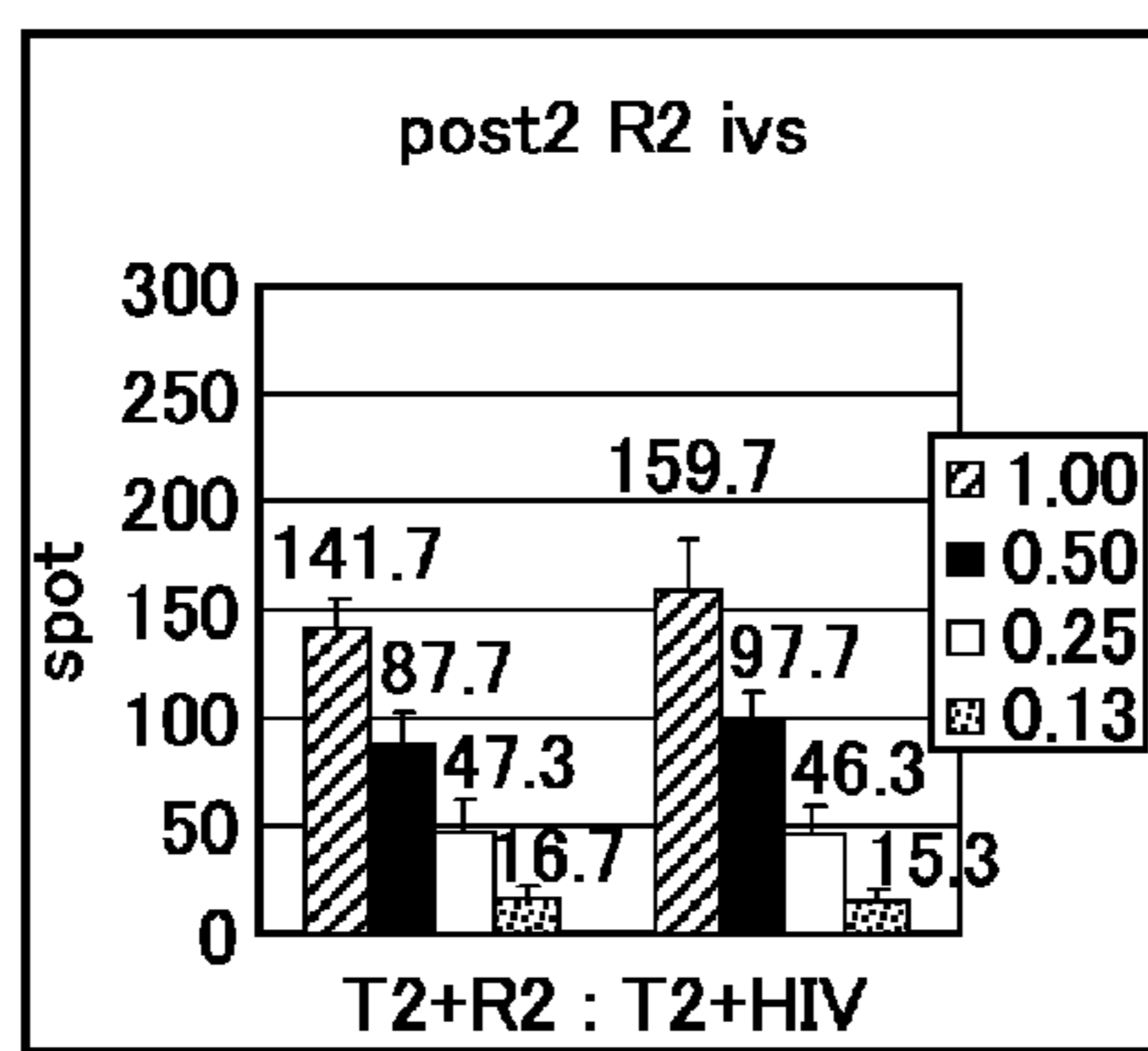
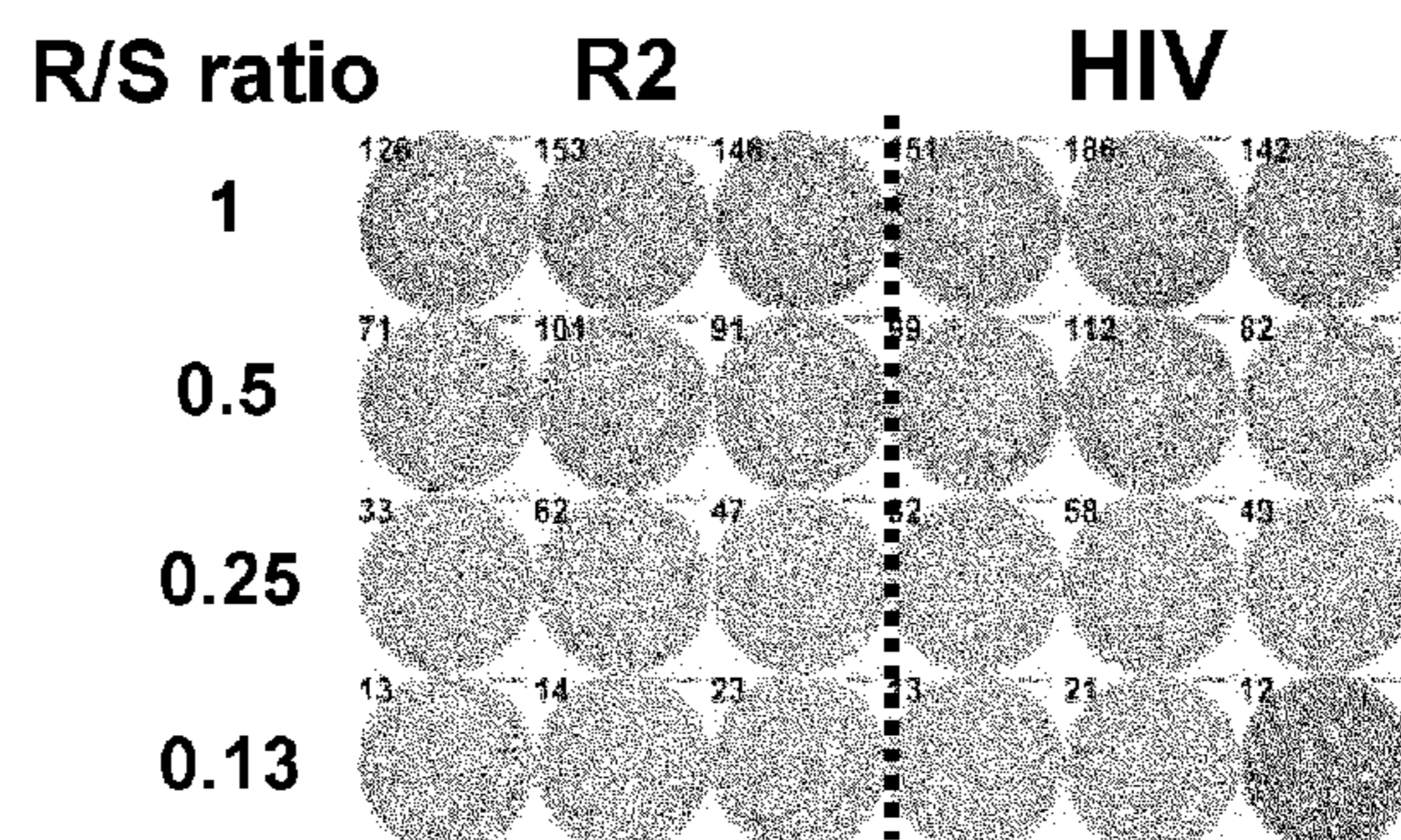


Fig. 5-2

c A0201-Case 1. post-2course (VEGFR2)



d A0201-Case 1. post-3course (VEGFR2)

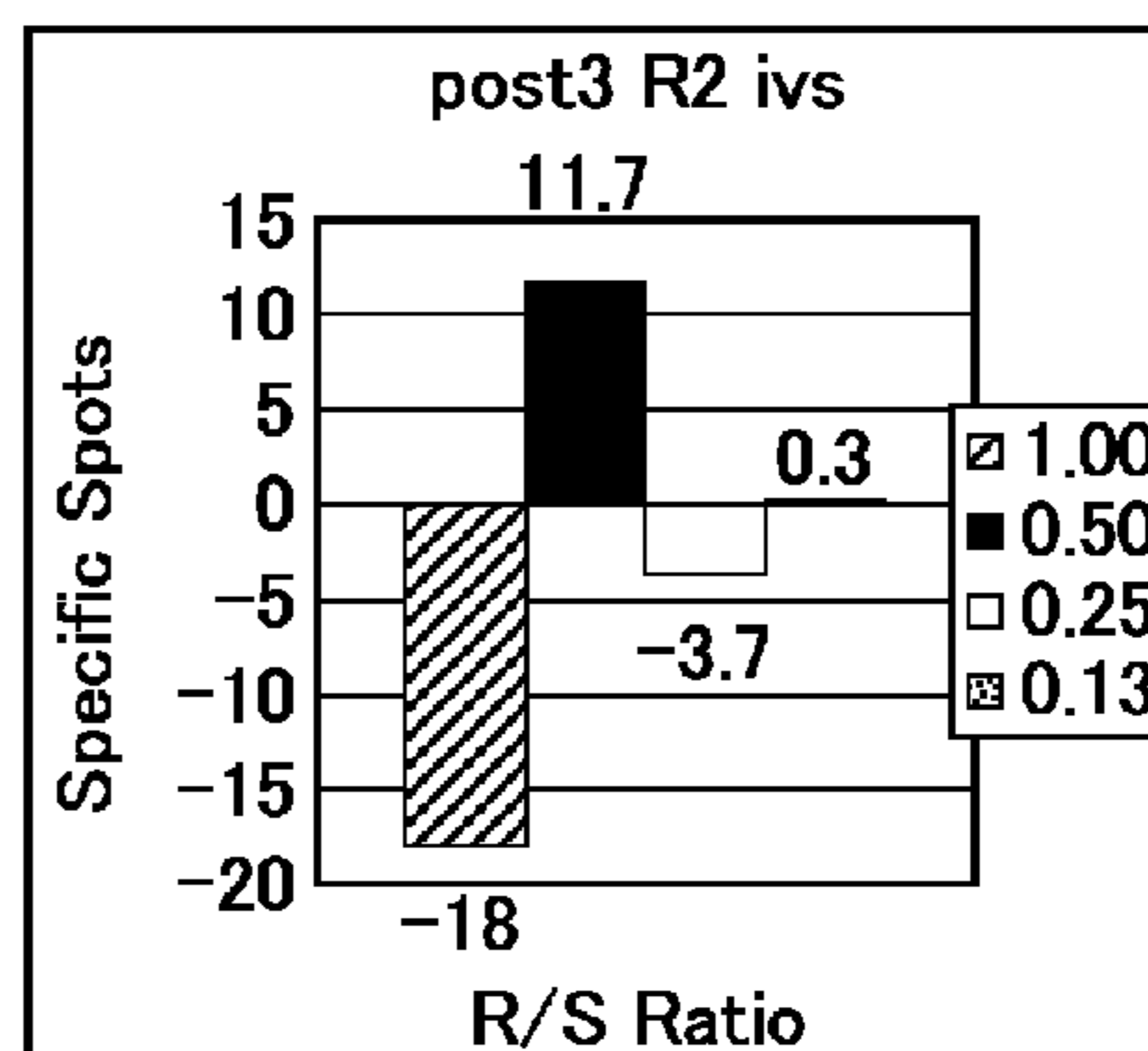
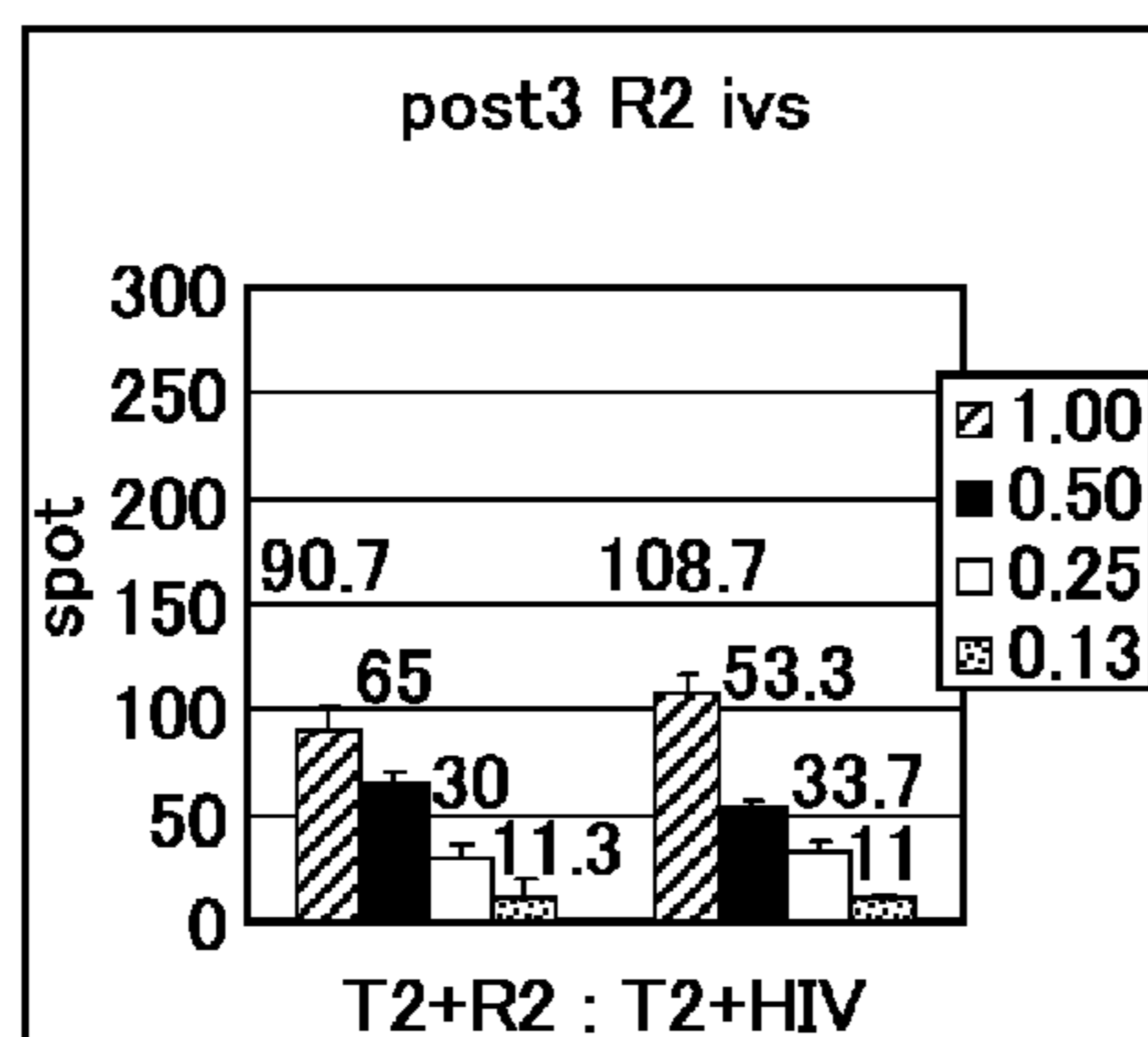
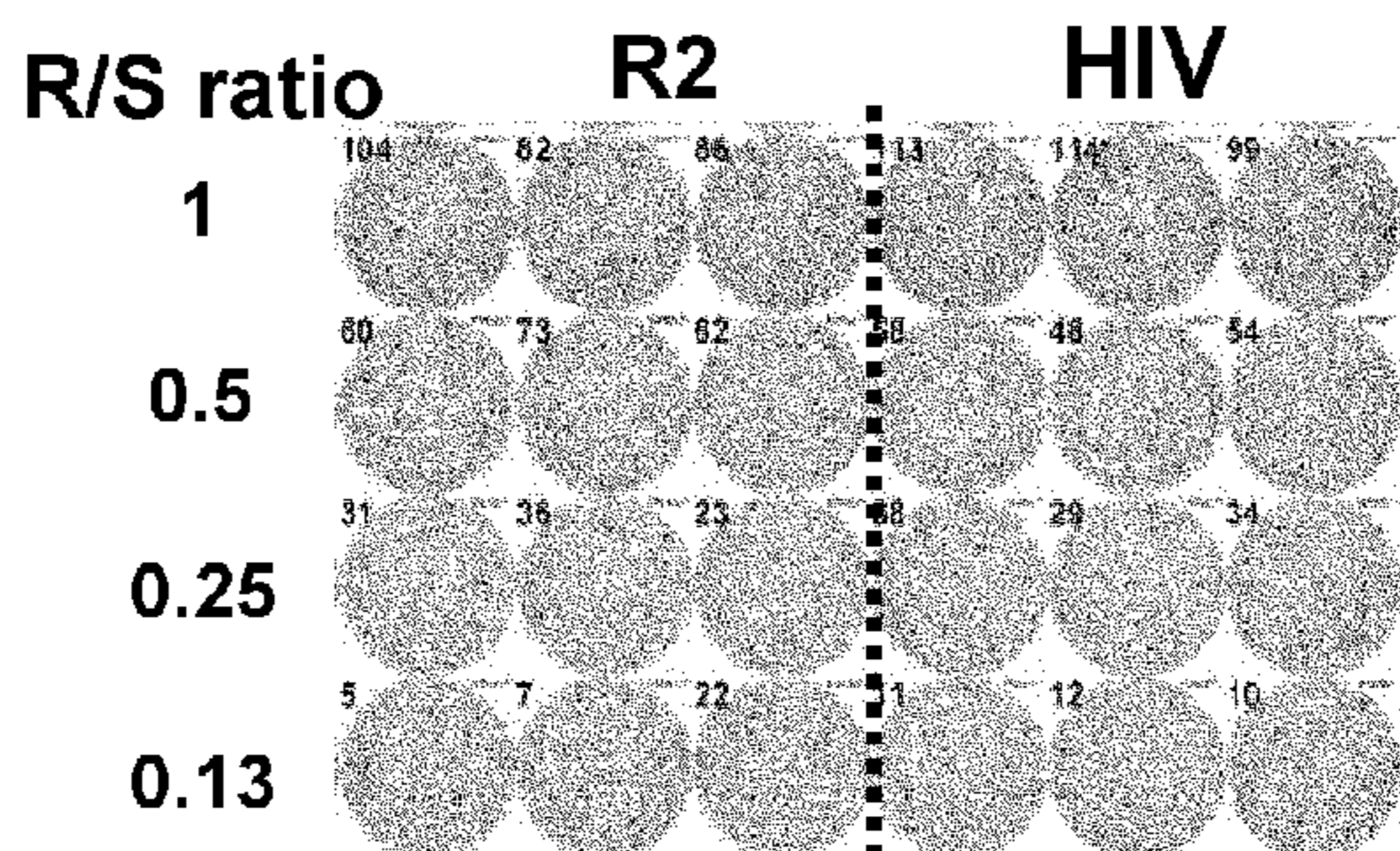


Fig. 5-3

e A0201-Case 1. post-4course (VEGFR2)

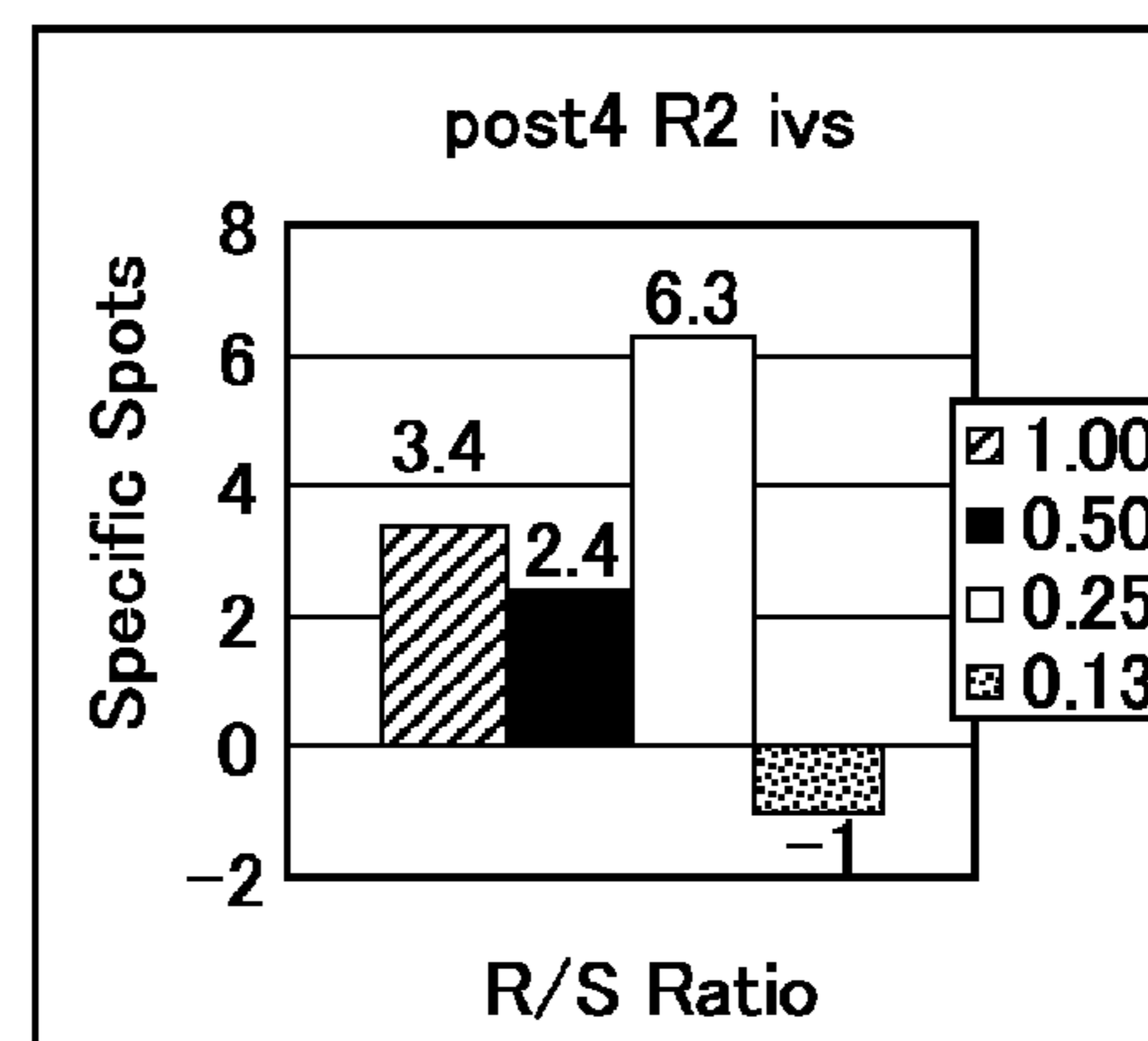
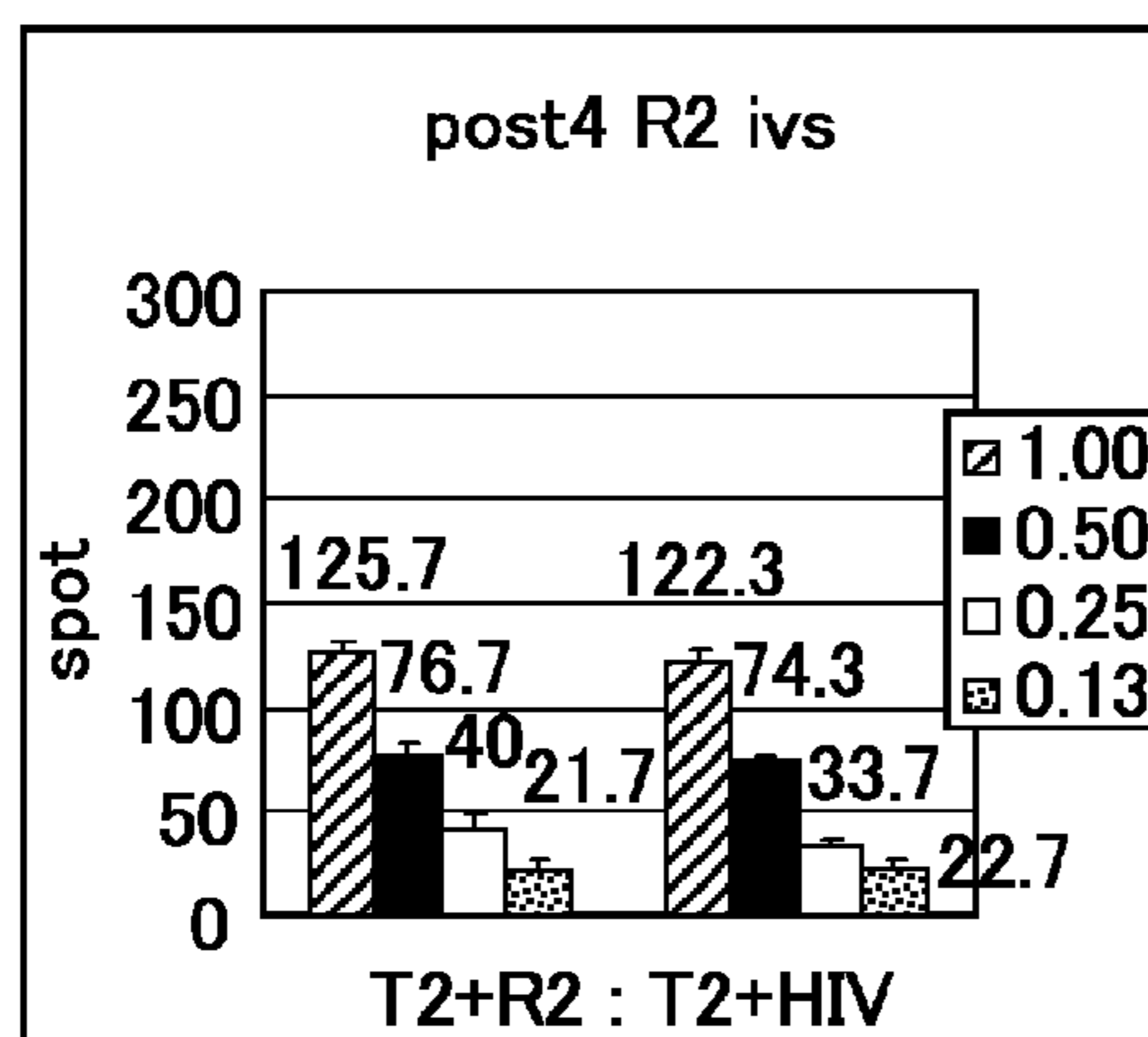
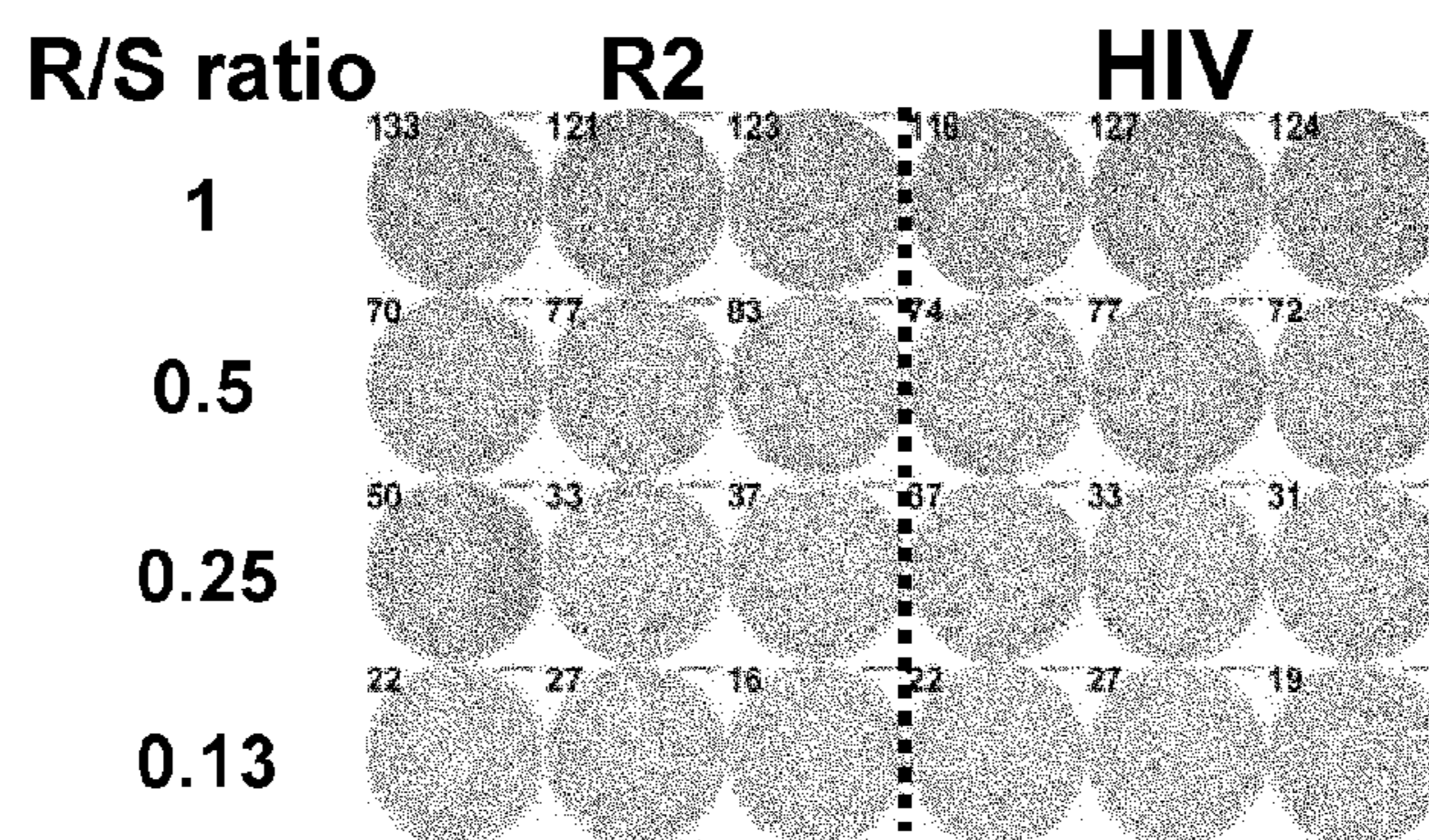
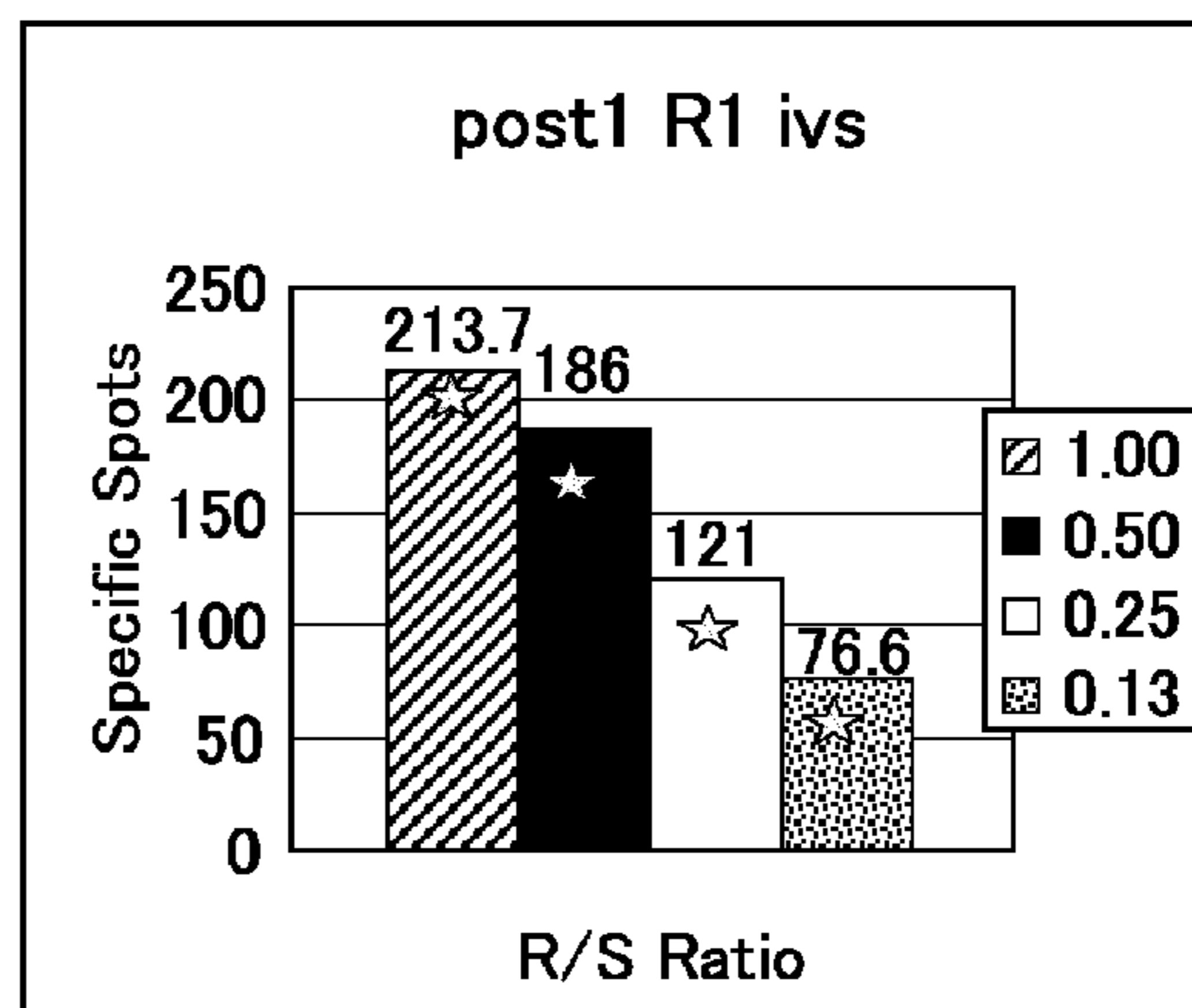
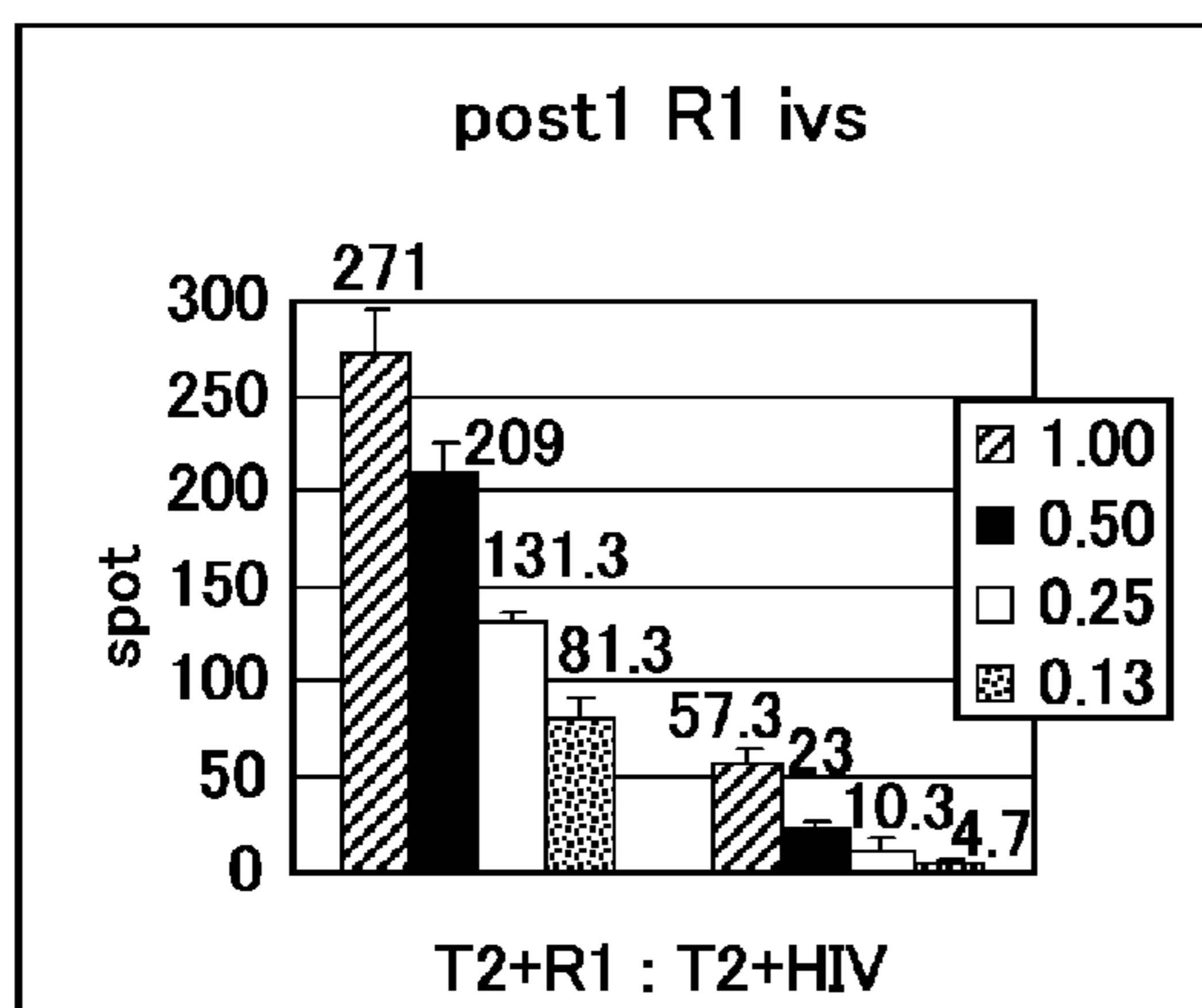
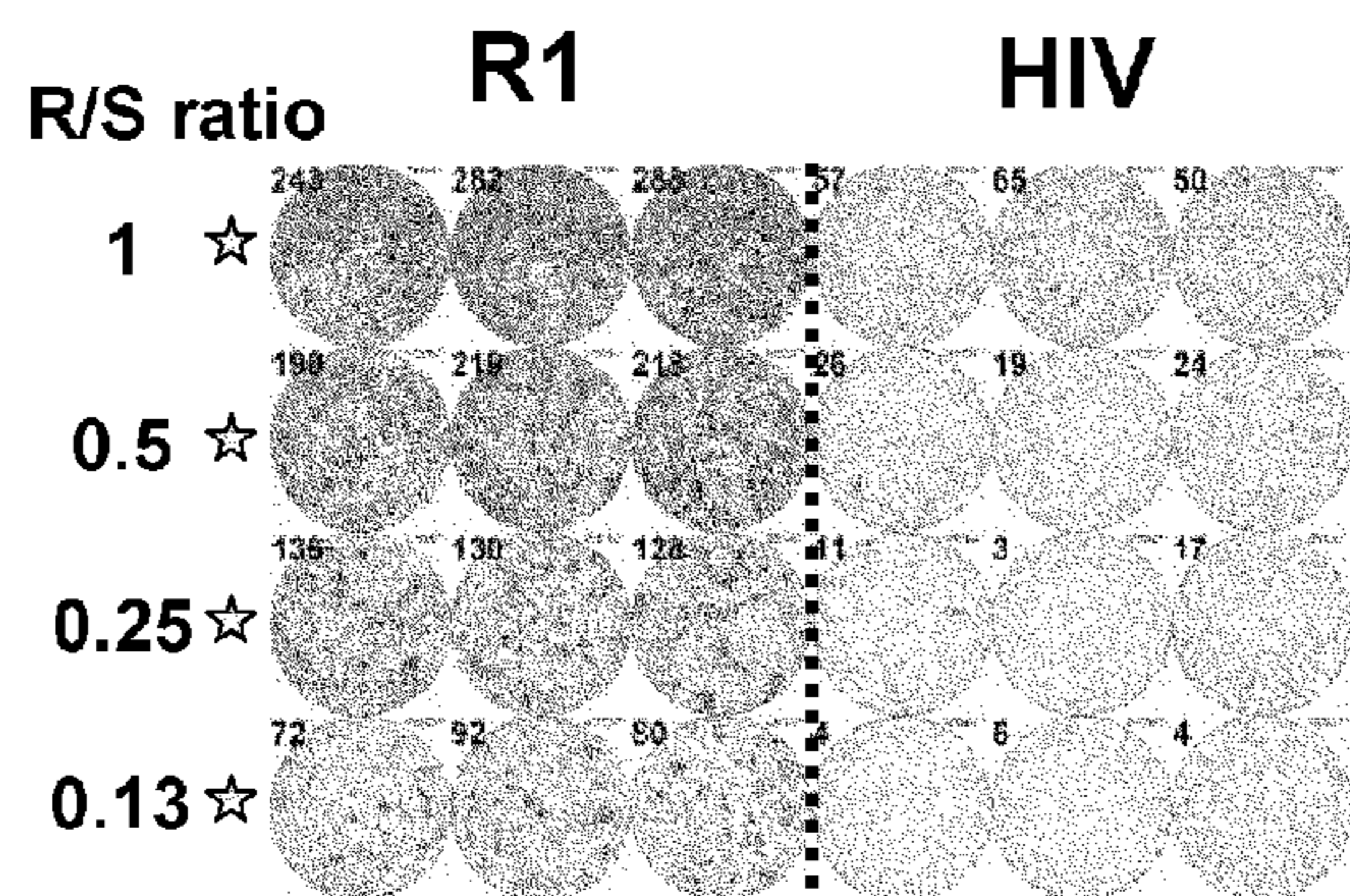
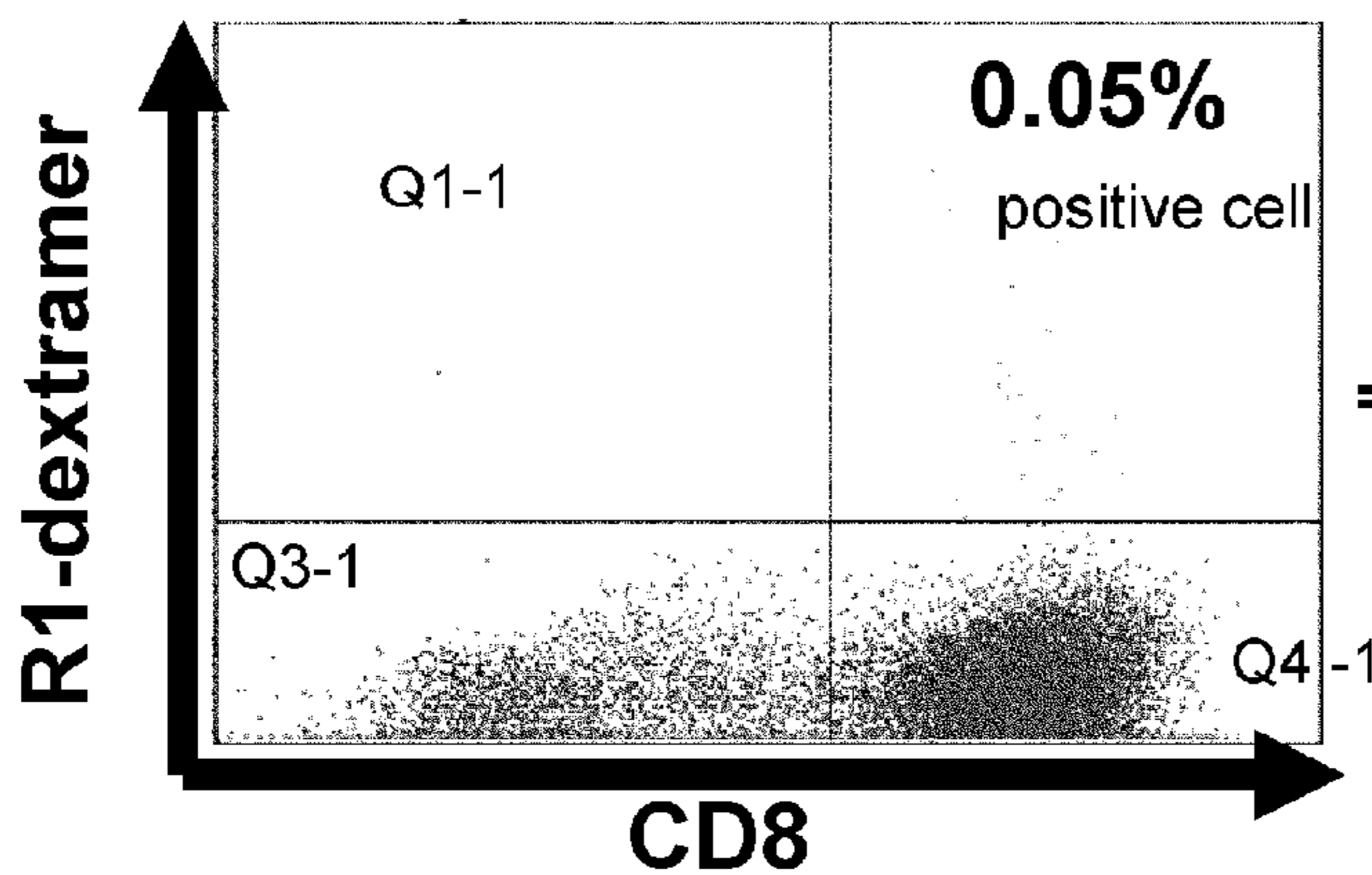


Fig. 6-1

a A0201-Case 3. post-1course (VEGFR1)



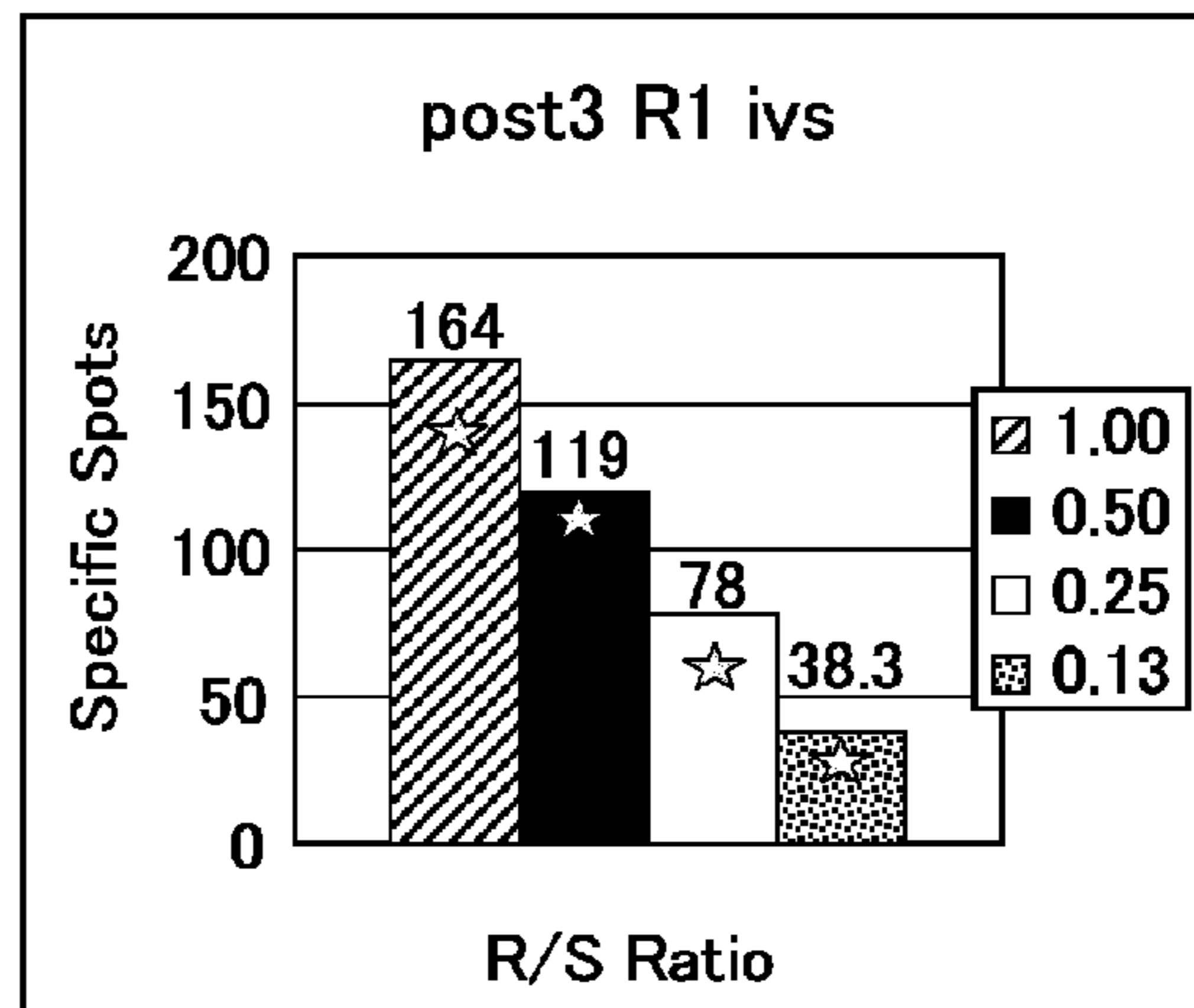
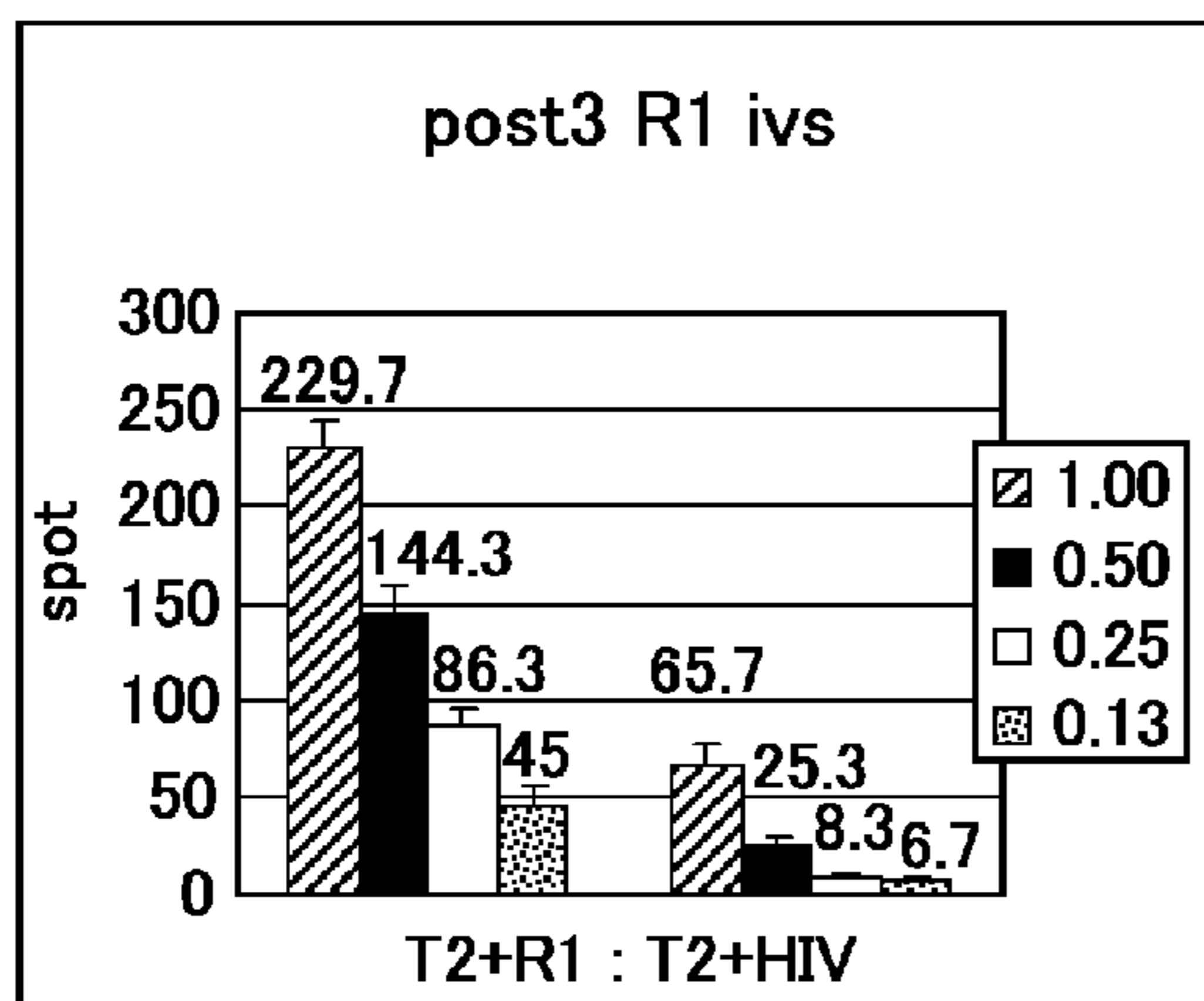
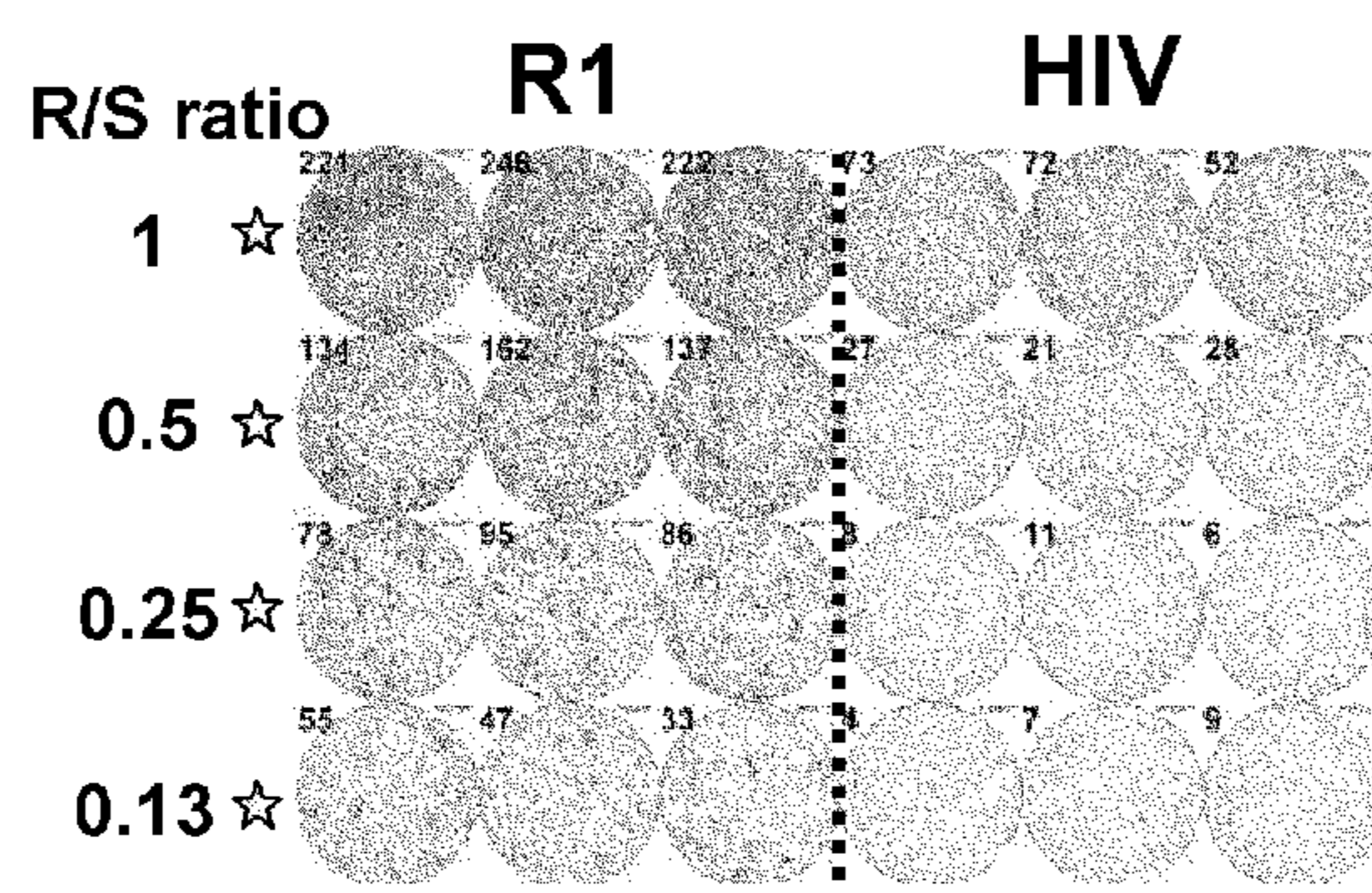
☆ P value <0.05



positive cell
= CD8+ R1 dextramer+/
CD3+CD4- (%)

Fig. 6-2

b A0201-Case 3. post-3course (VEGFR1)



☆ P value <0.05

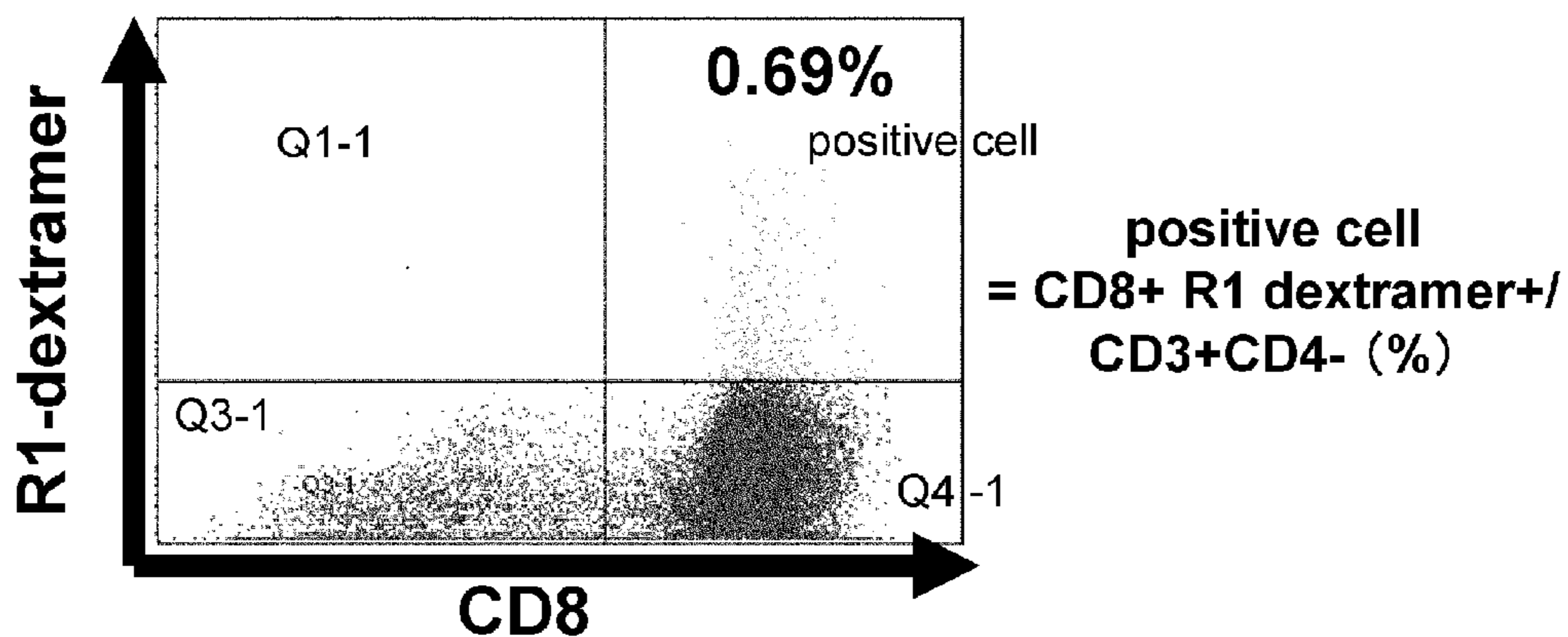
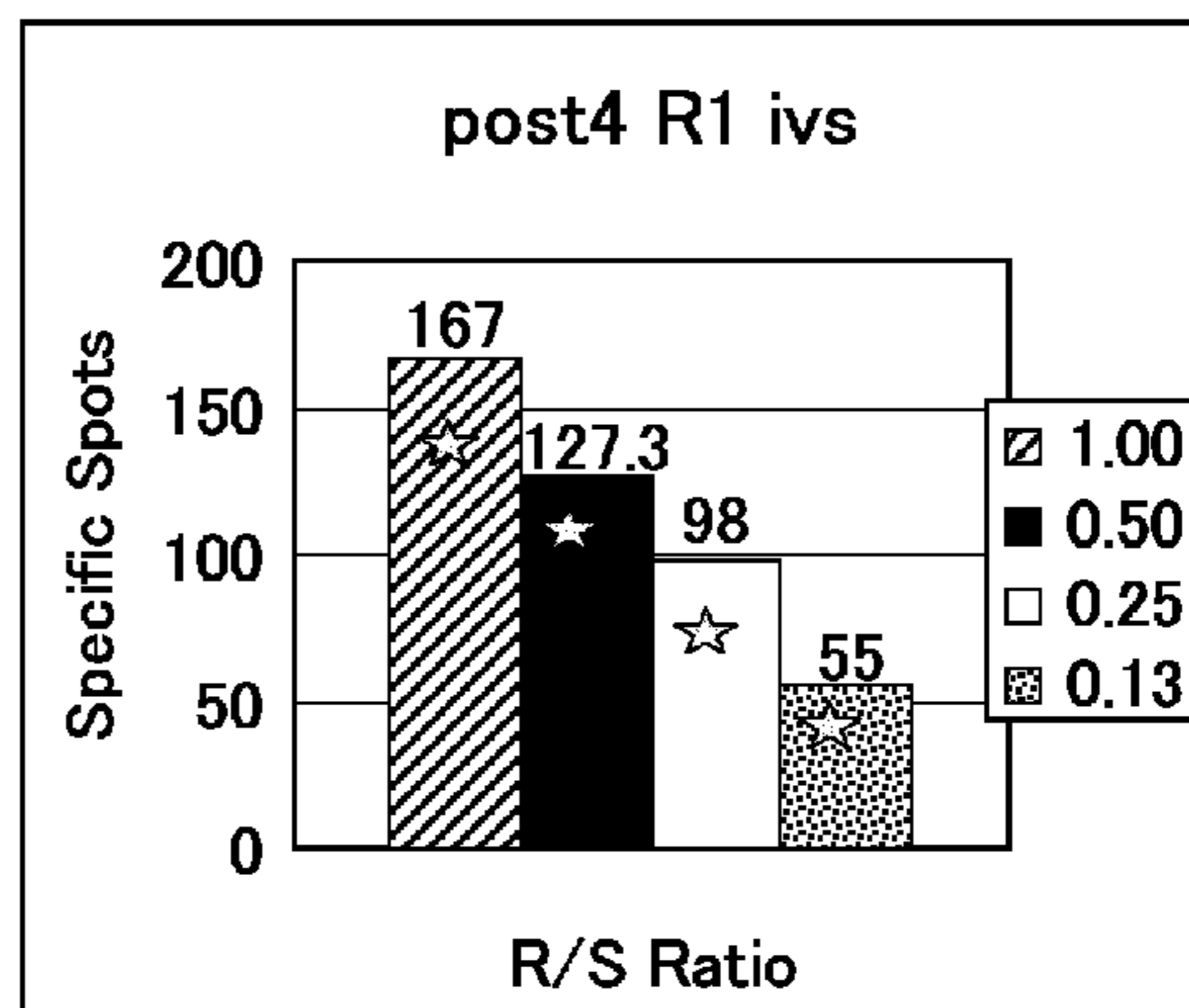
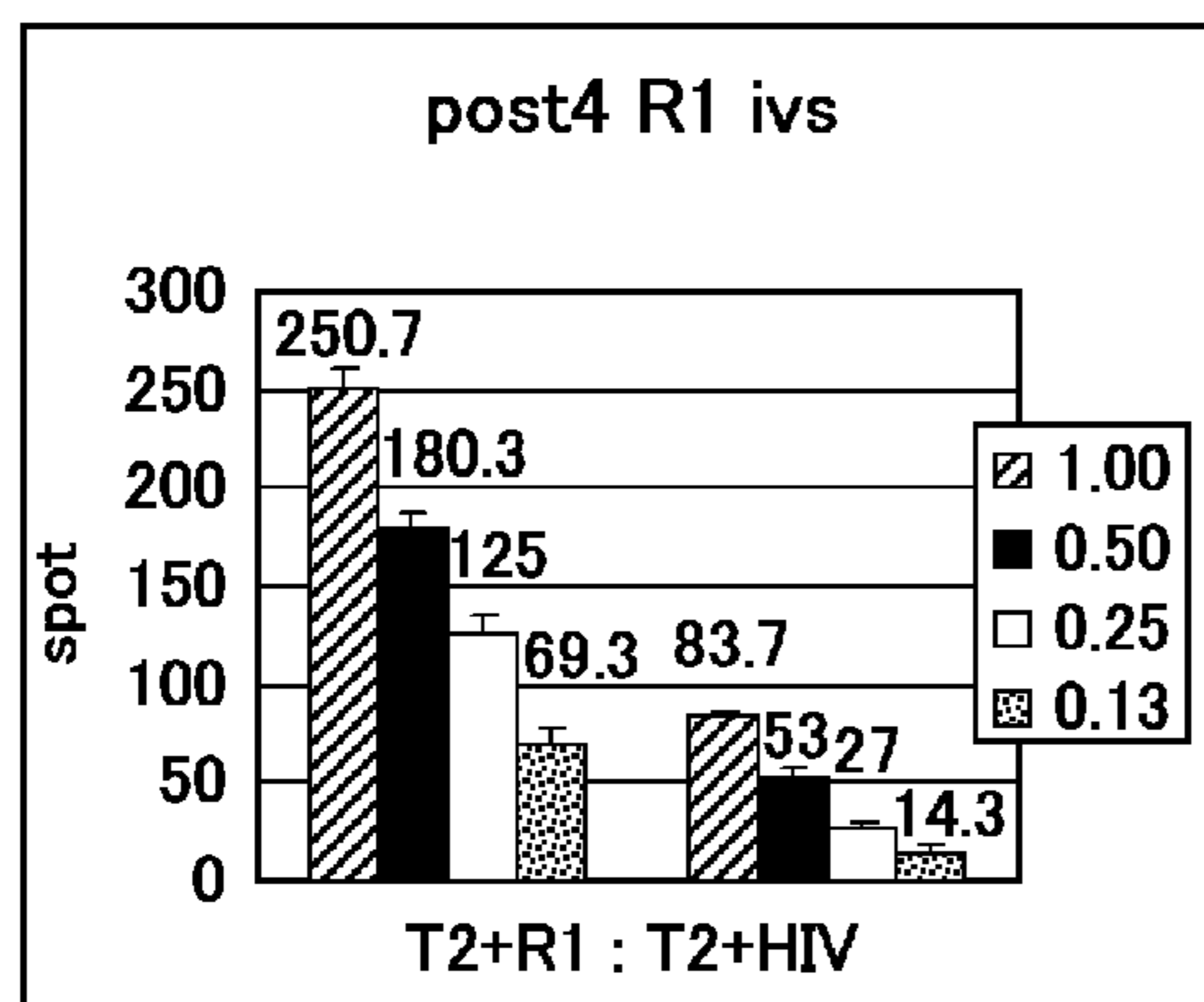
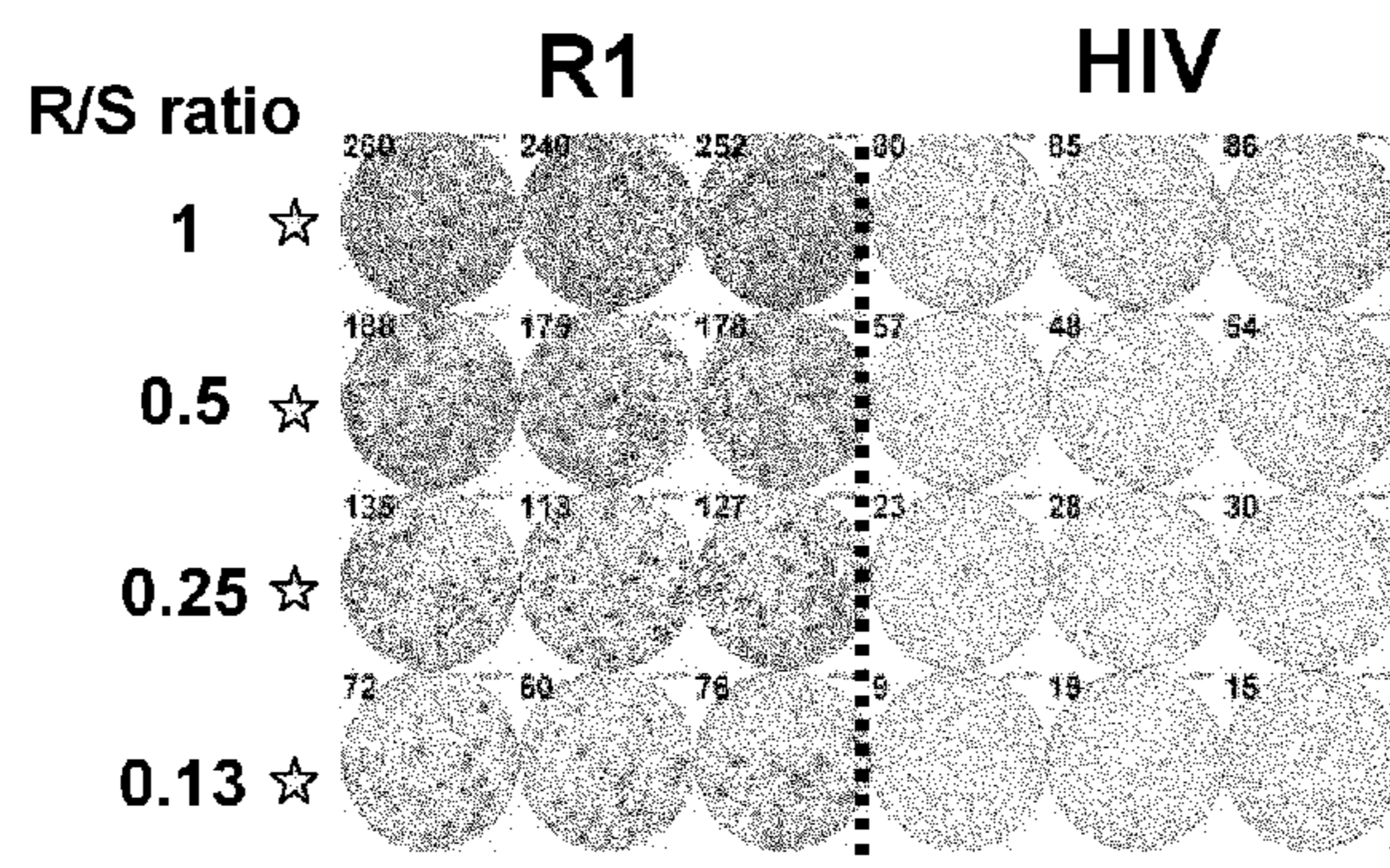
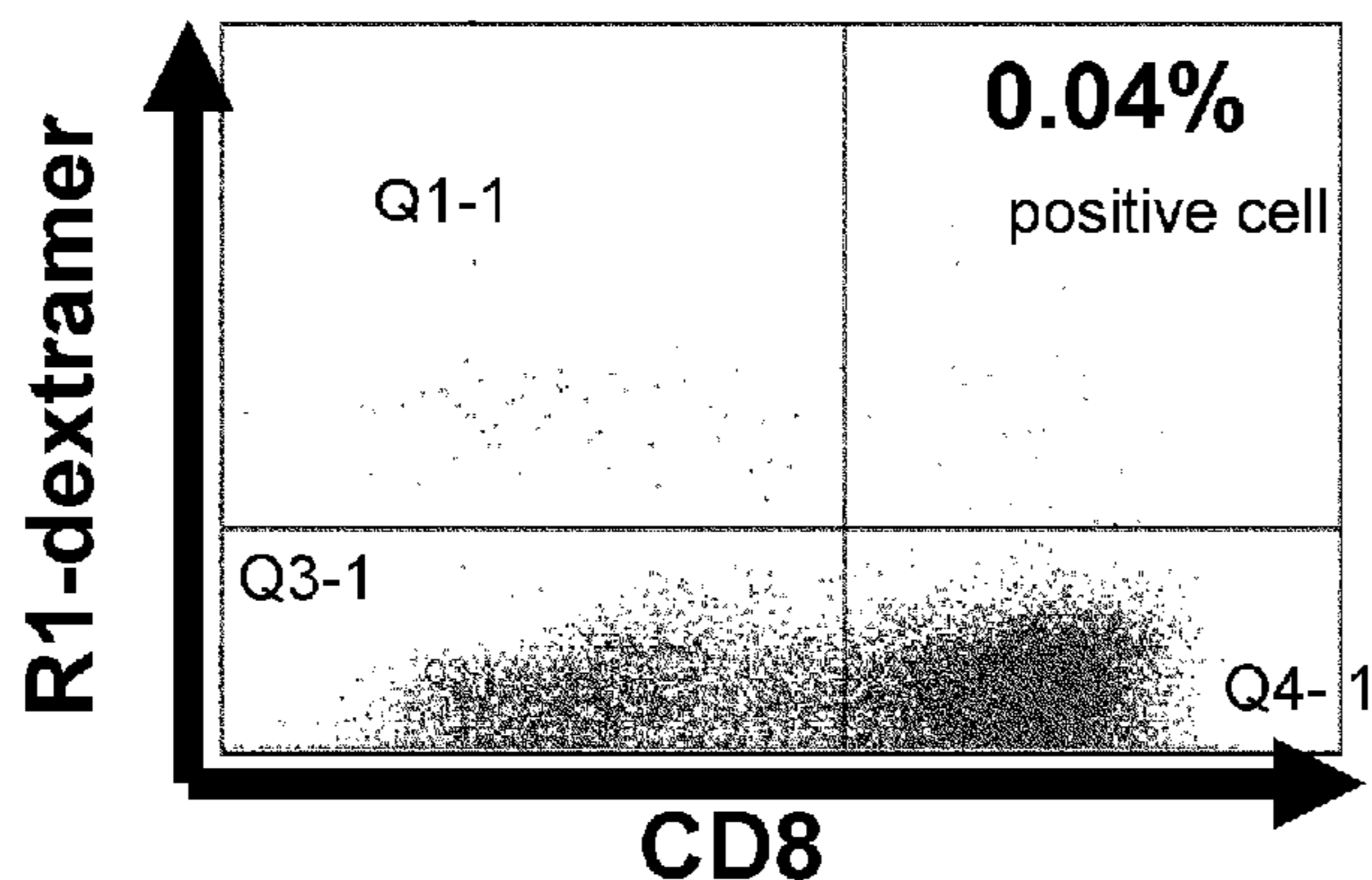


Fig. 6-3

c A0201-Case 3. post-4course (VEGFR1)



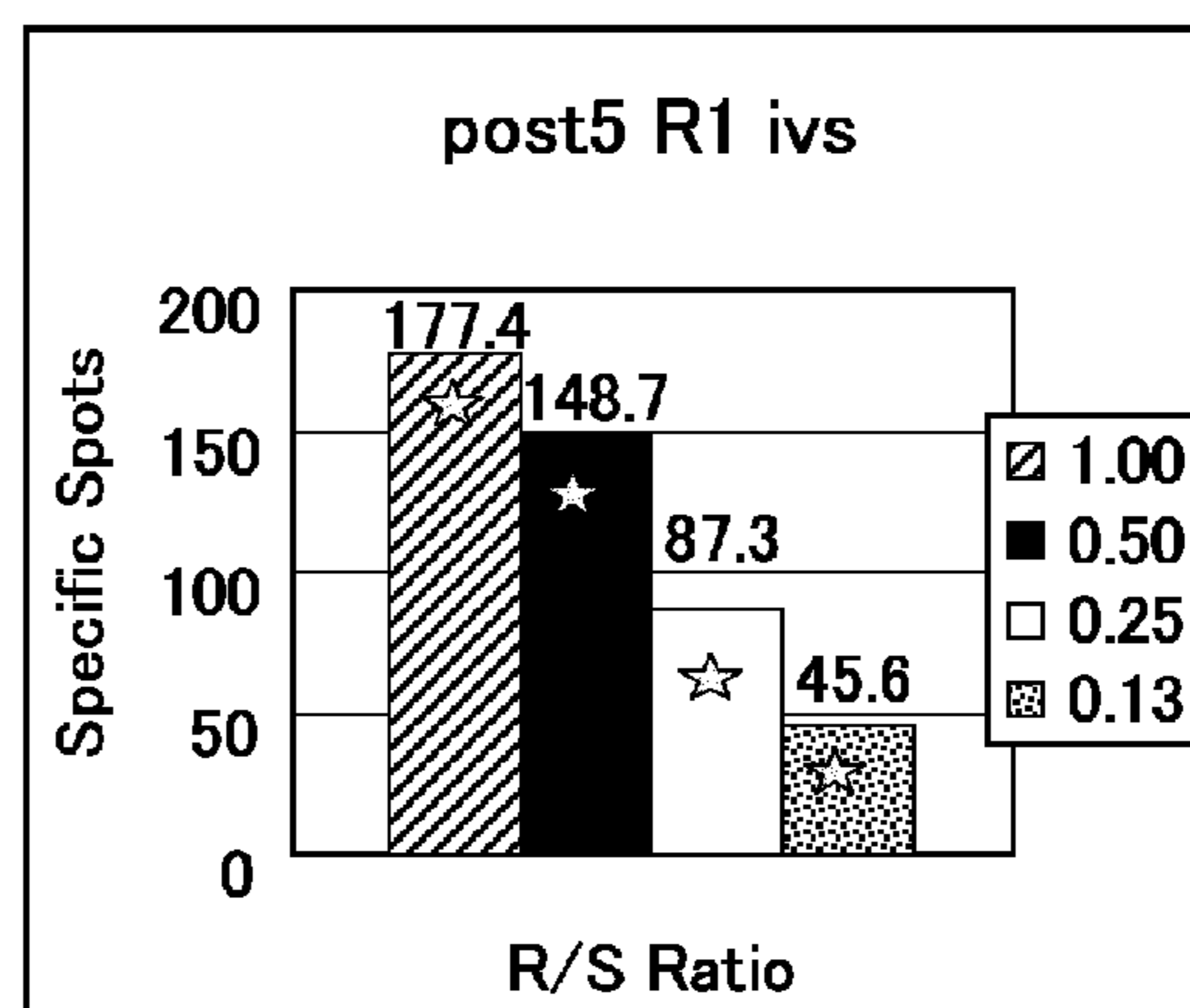
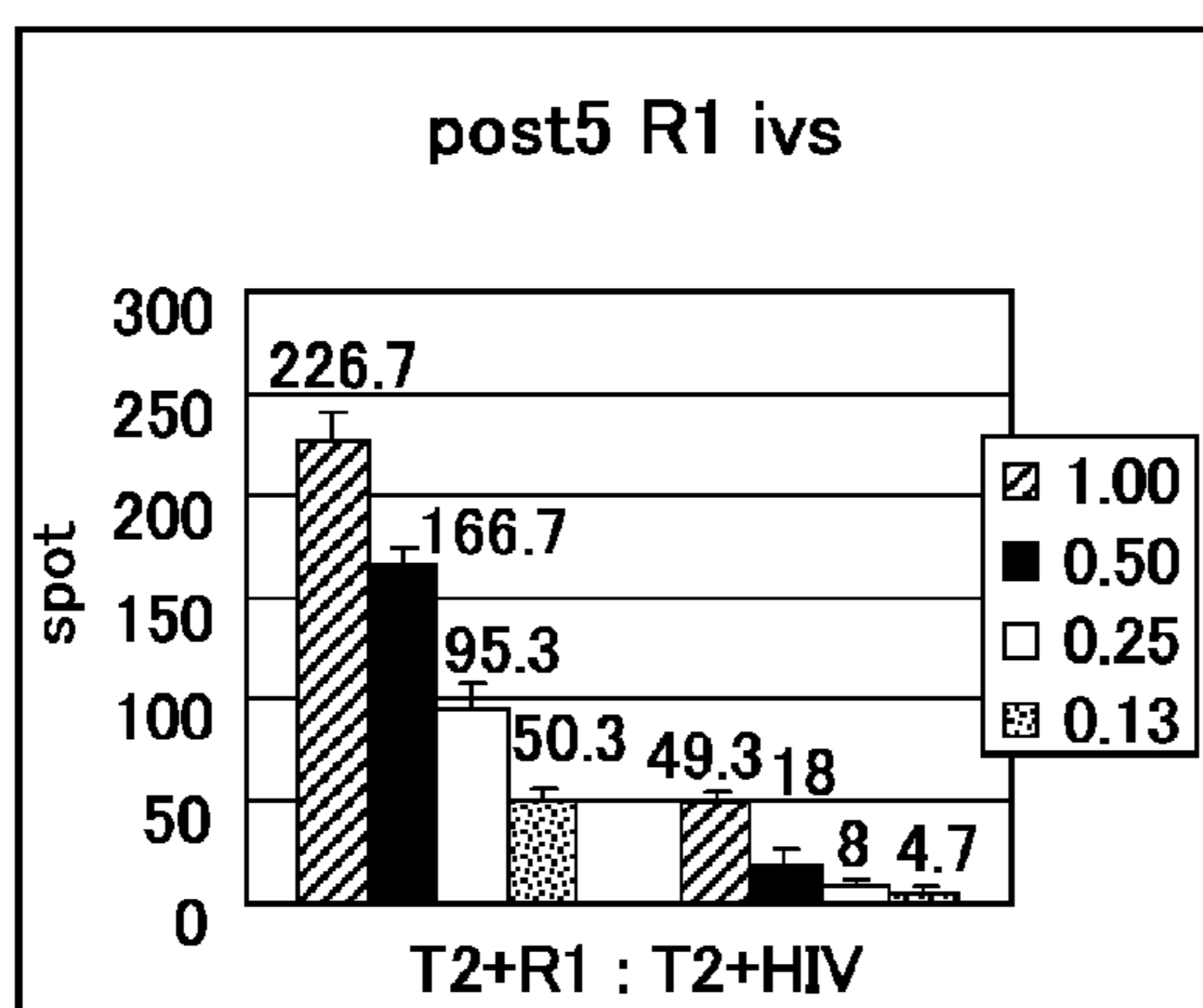
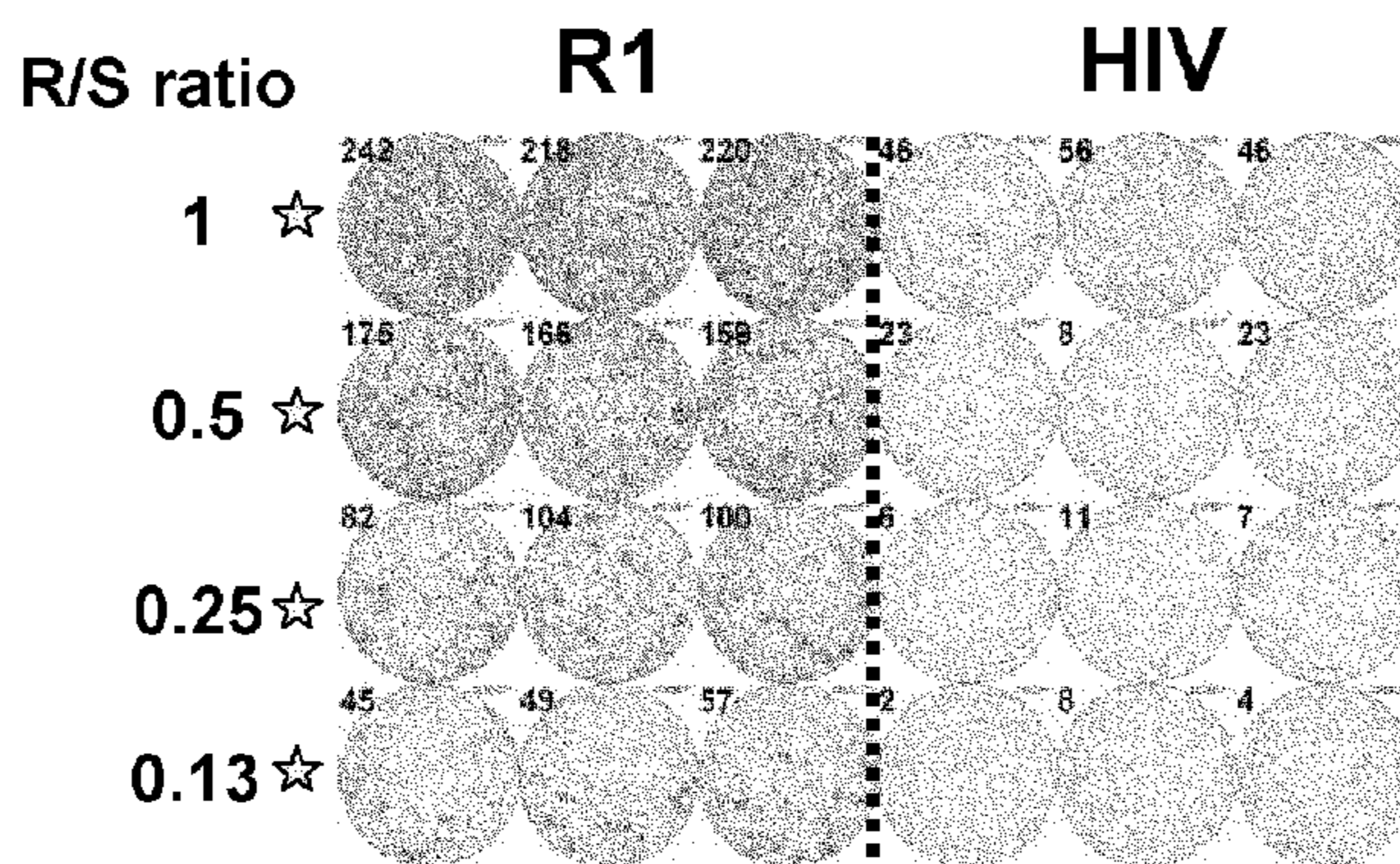
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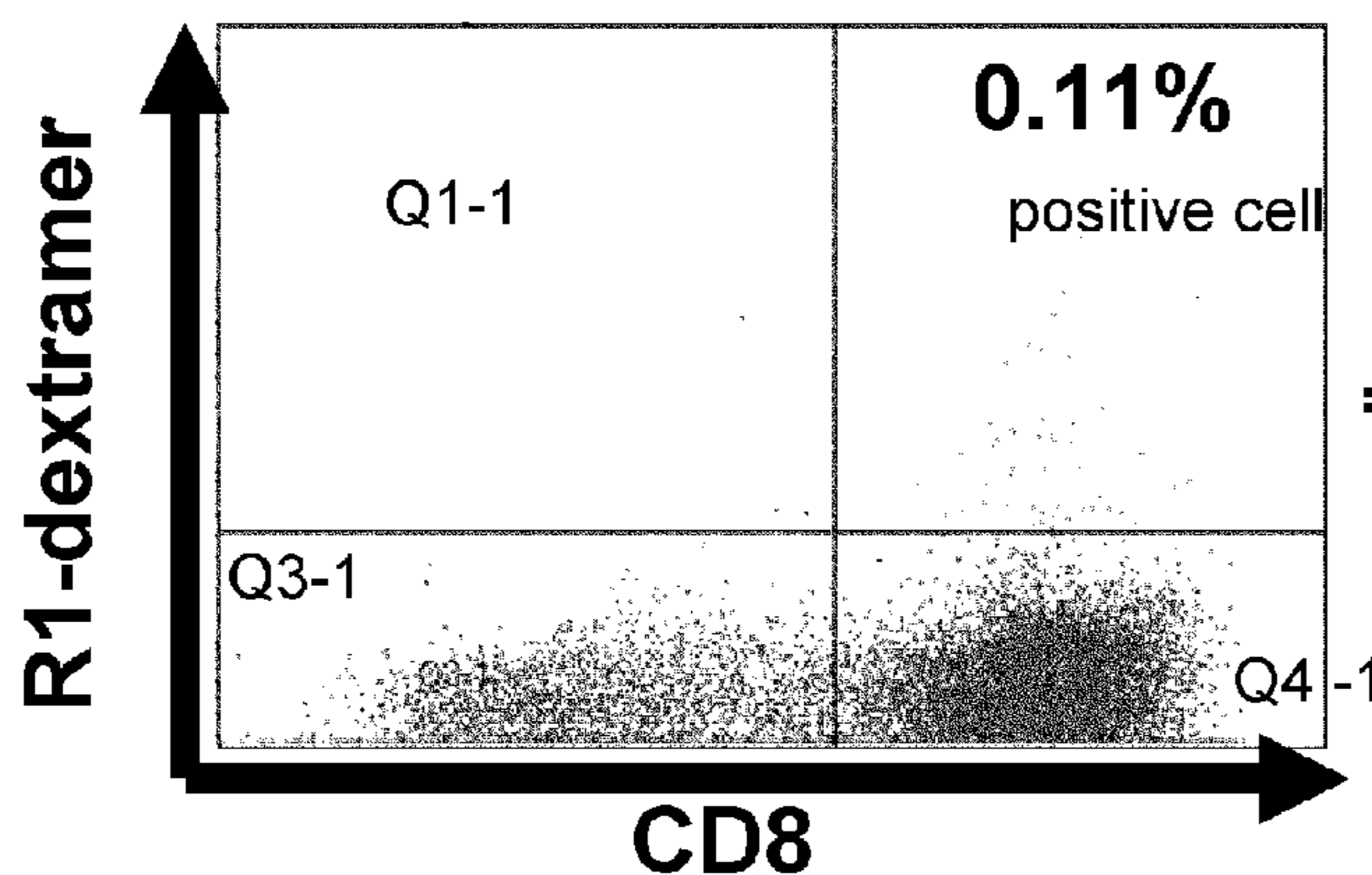
positive cell
= CD8+ R1 dextramer+/
CD3+CD4- (%)

Fig. 6-4

d A0201-Case 3. post-5 course (VEGFR1)



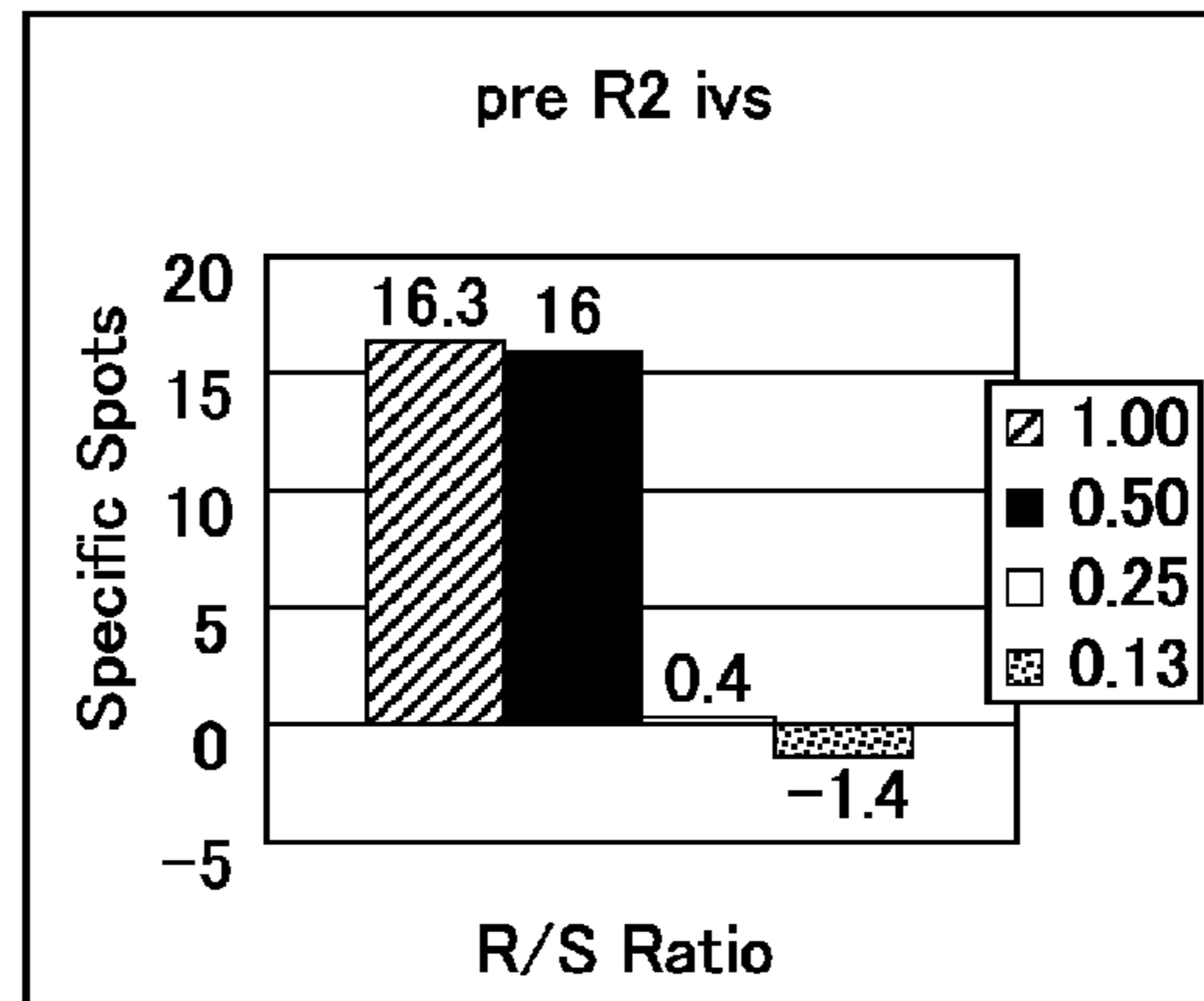
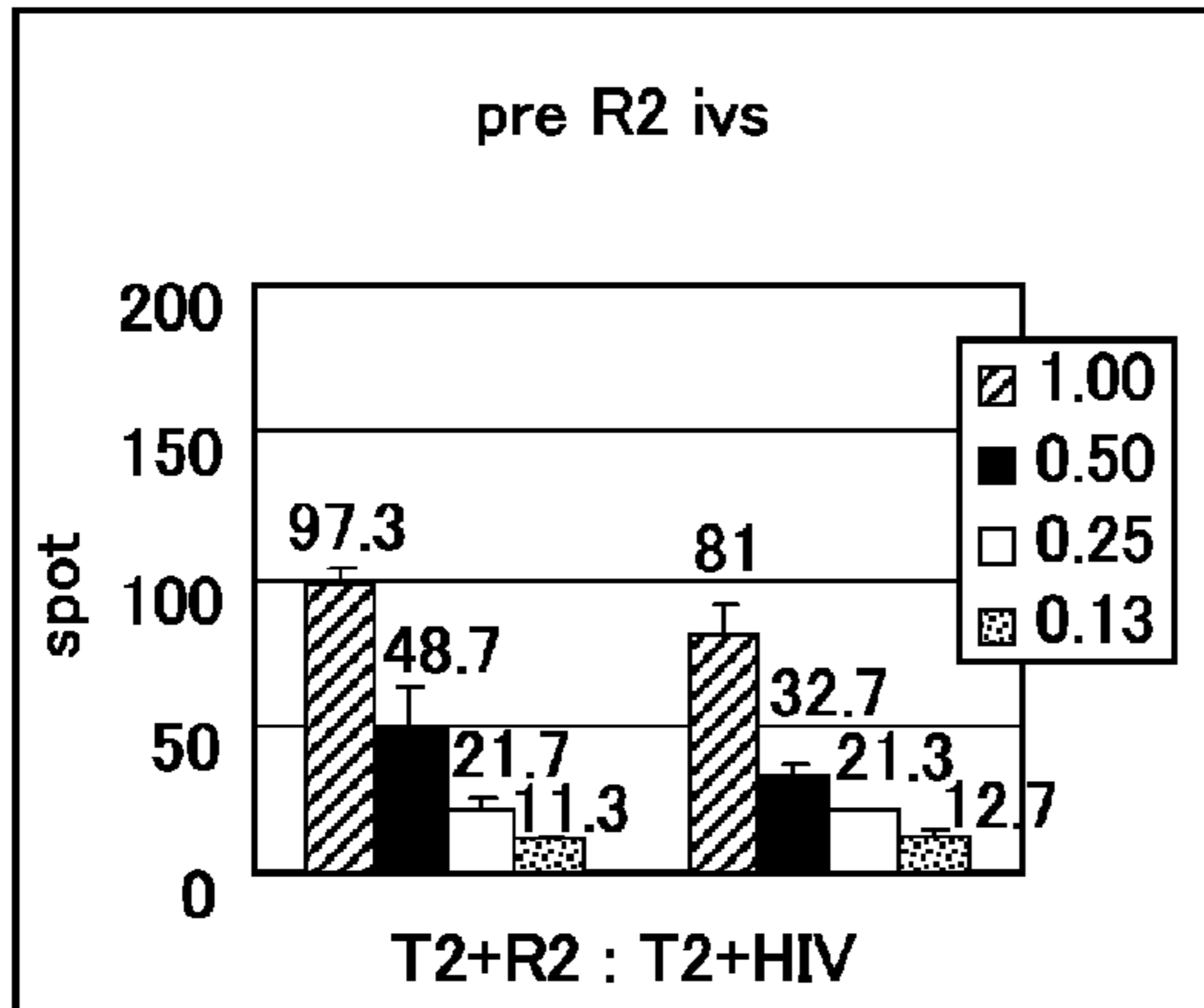
☆ P value <0.05



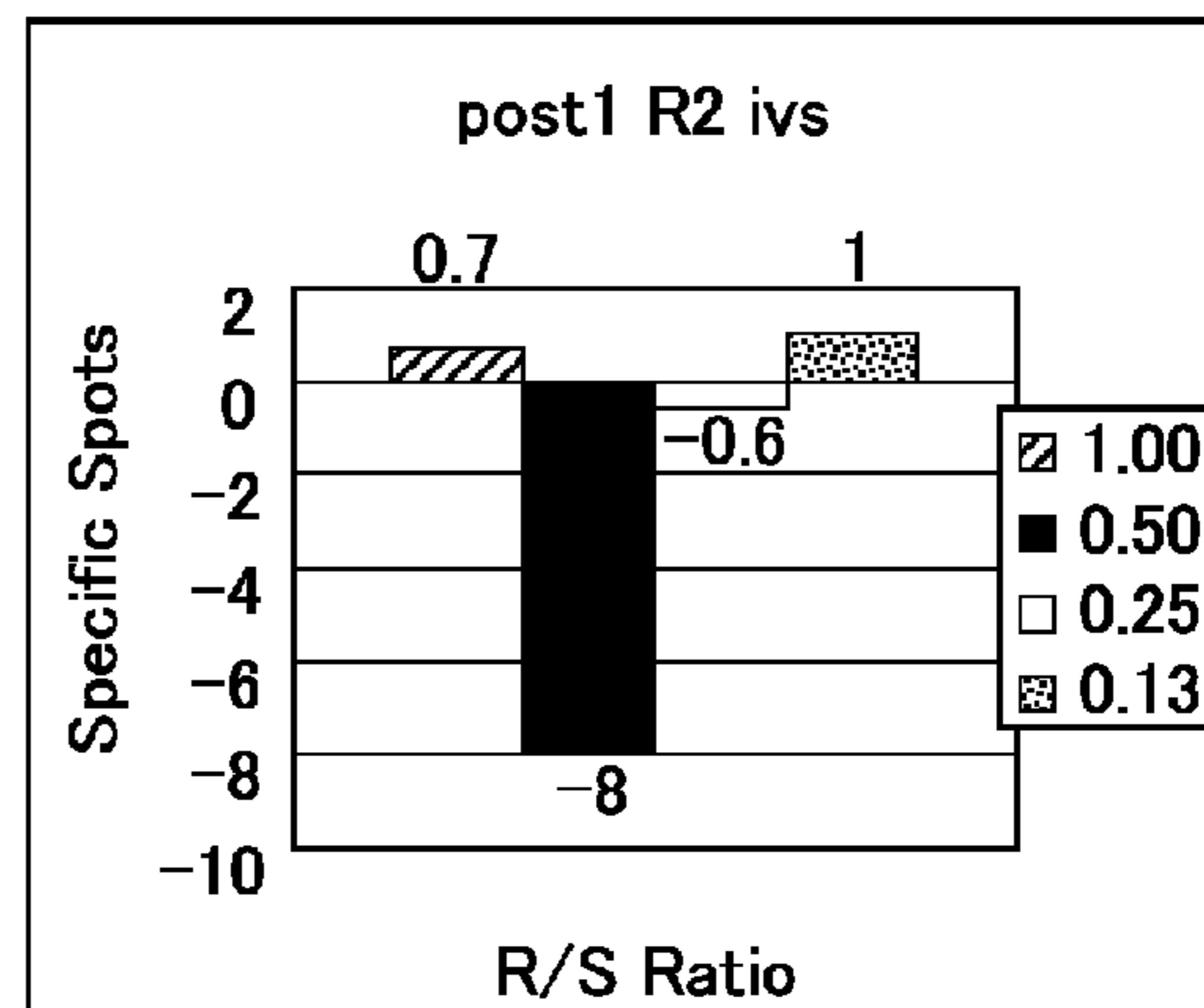
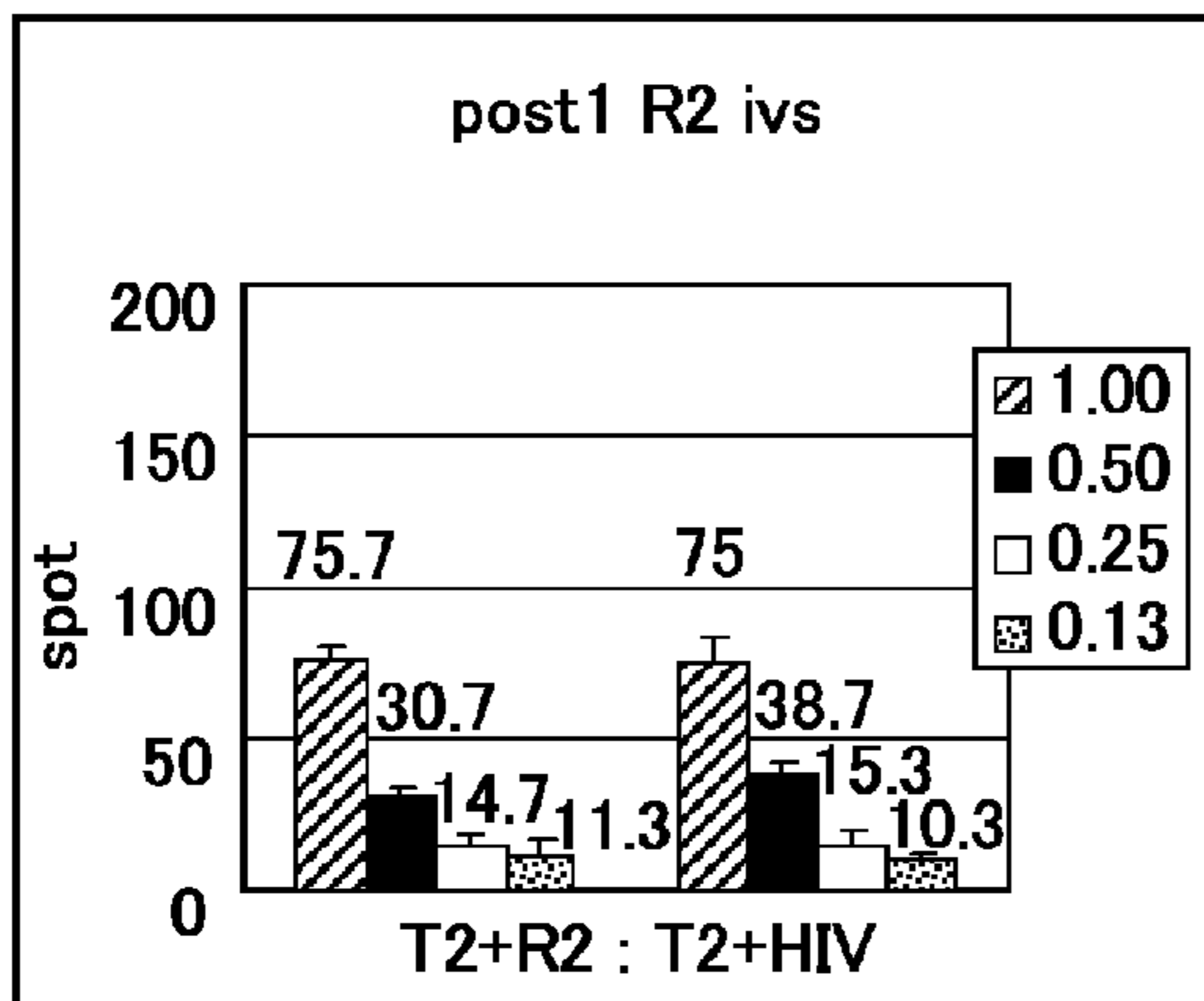
positive cell
= CD8+ R1 dextramer+/
CD3+CD4- (%)

Fig. 7-1

a A0201-Case 3. pre-treatment (VEGFR2)



b A0201-Case 3. post-1course (VEGFR2)



c A0201-Case 3. post-3course (VEGFR2)

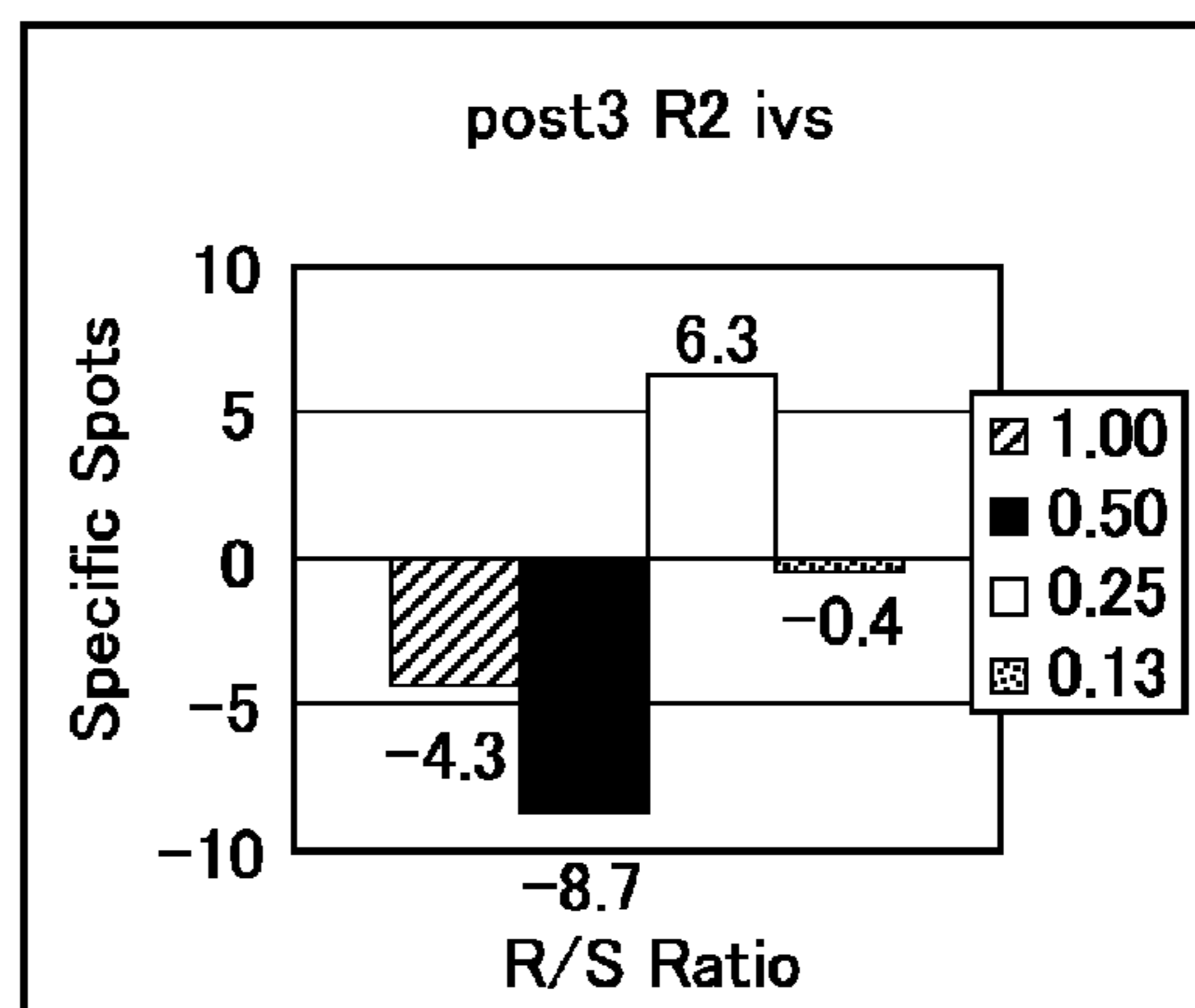
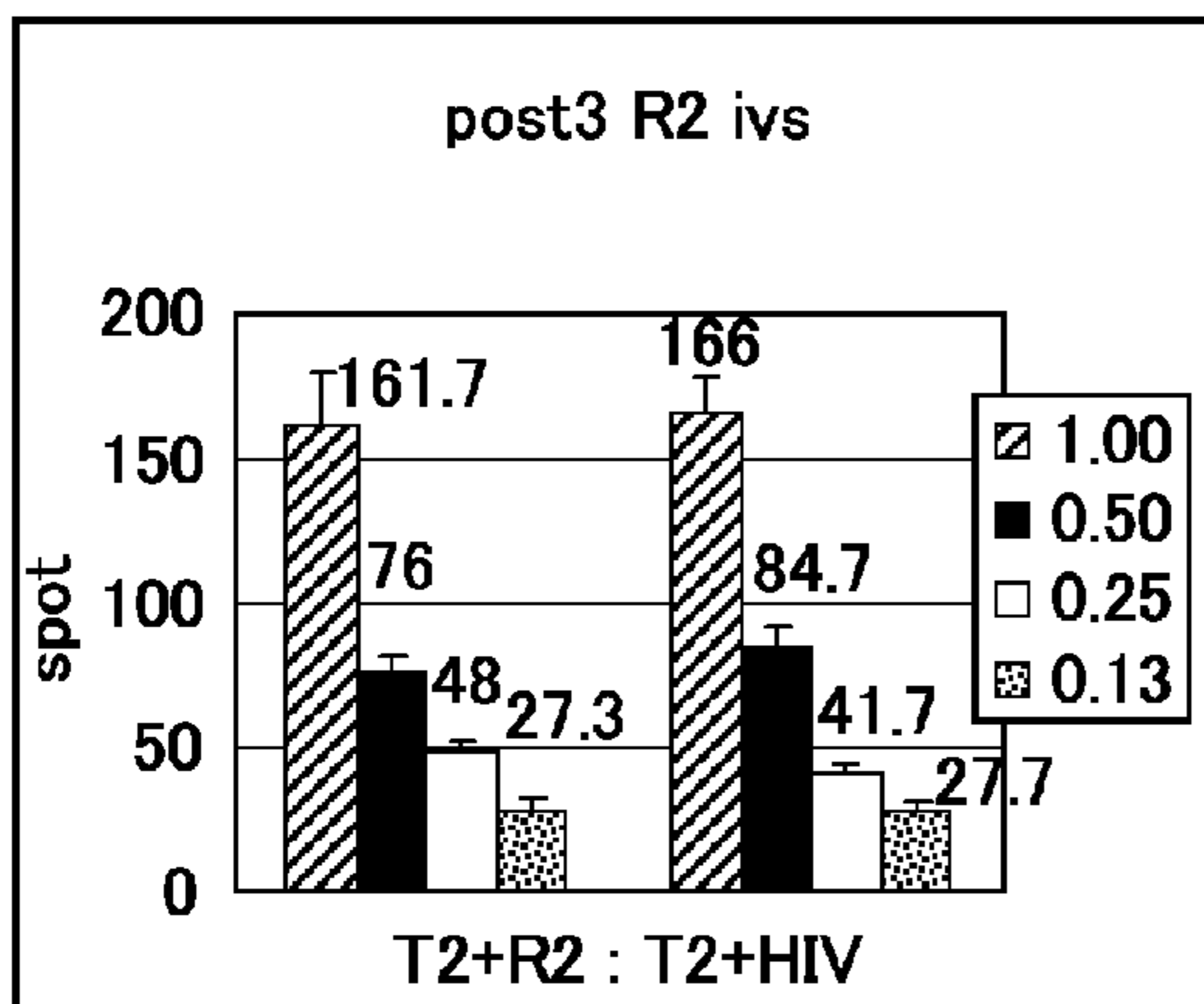
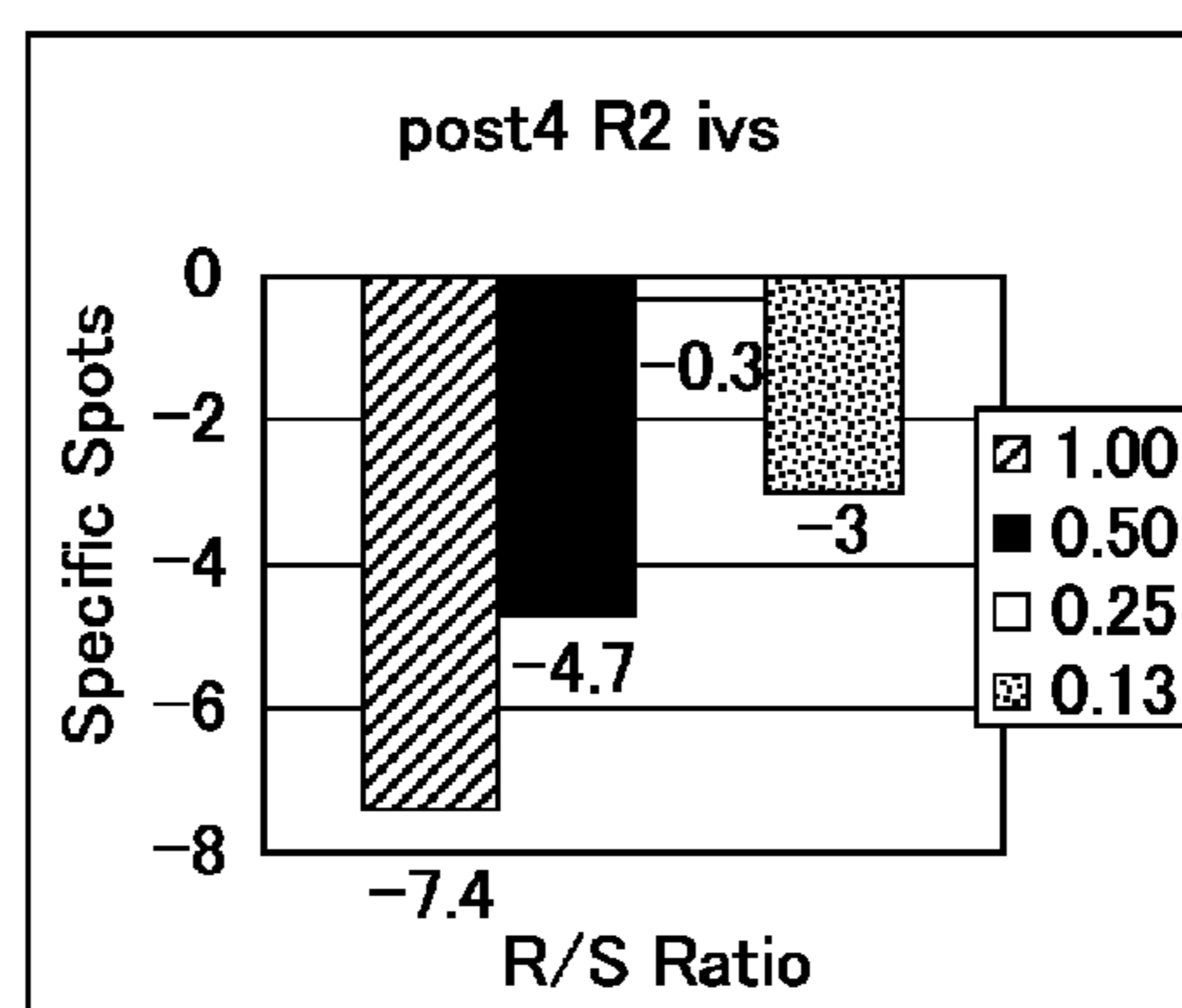
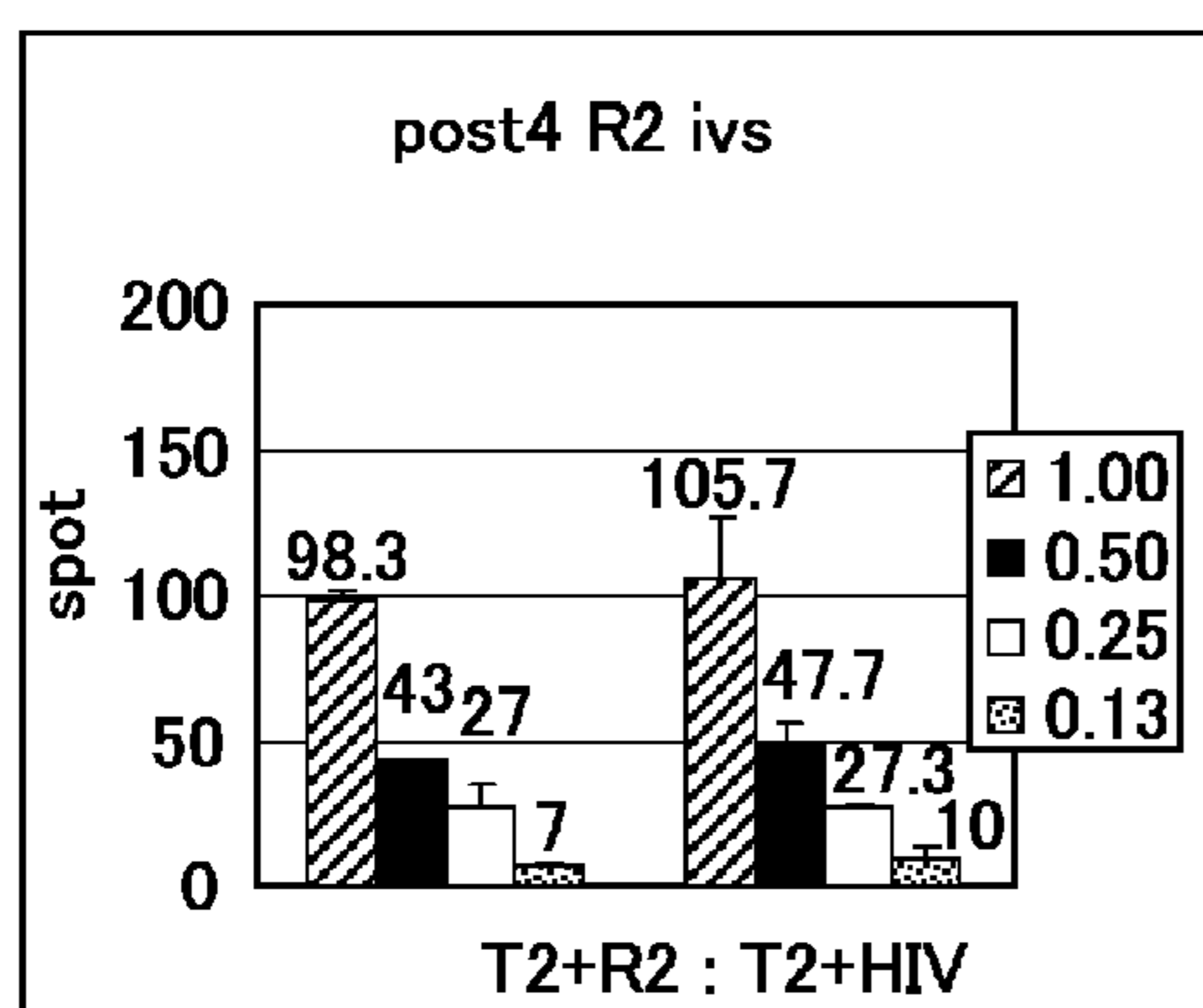


Fig. 7-2

d A0201-Case 3. post-4course (VEGFR2)



e A0201-Case 3. post-5course (VEGFR2)

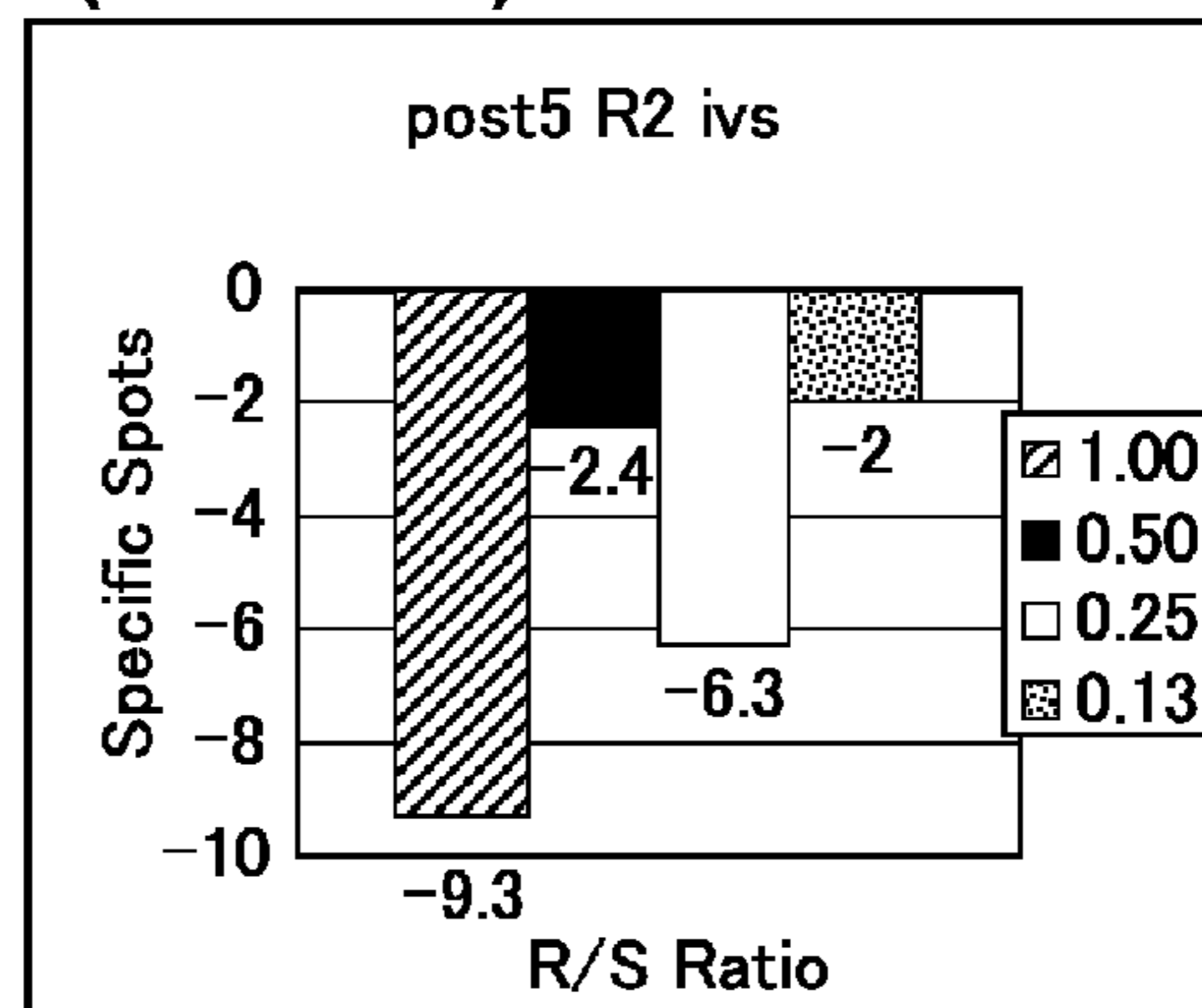
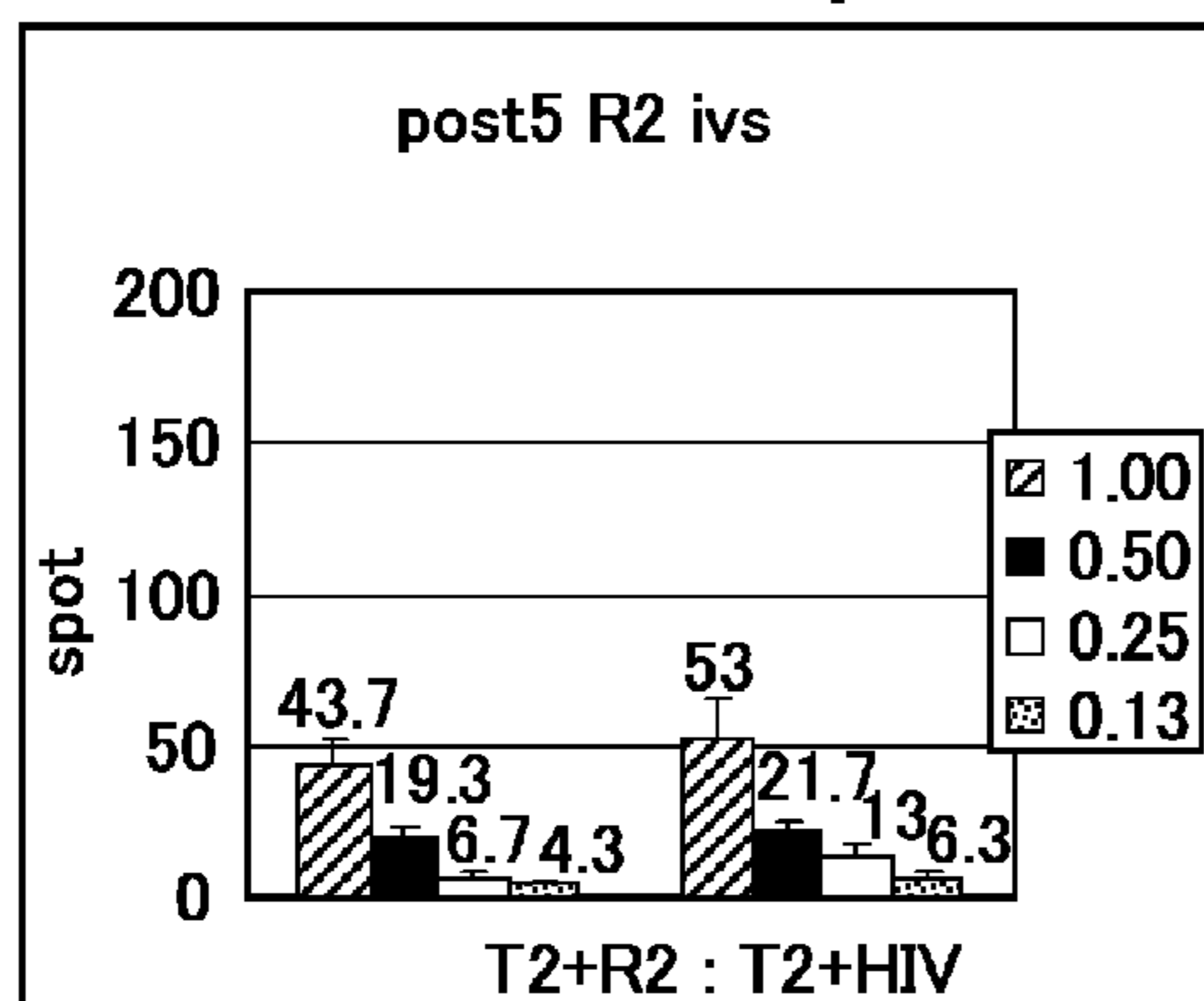
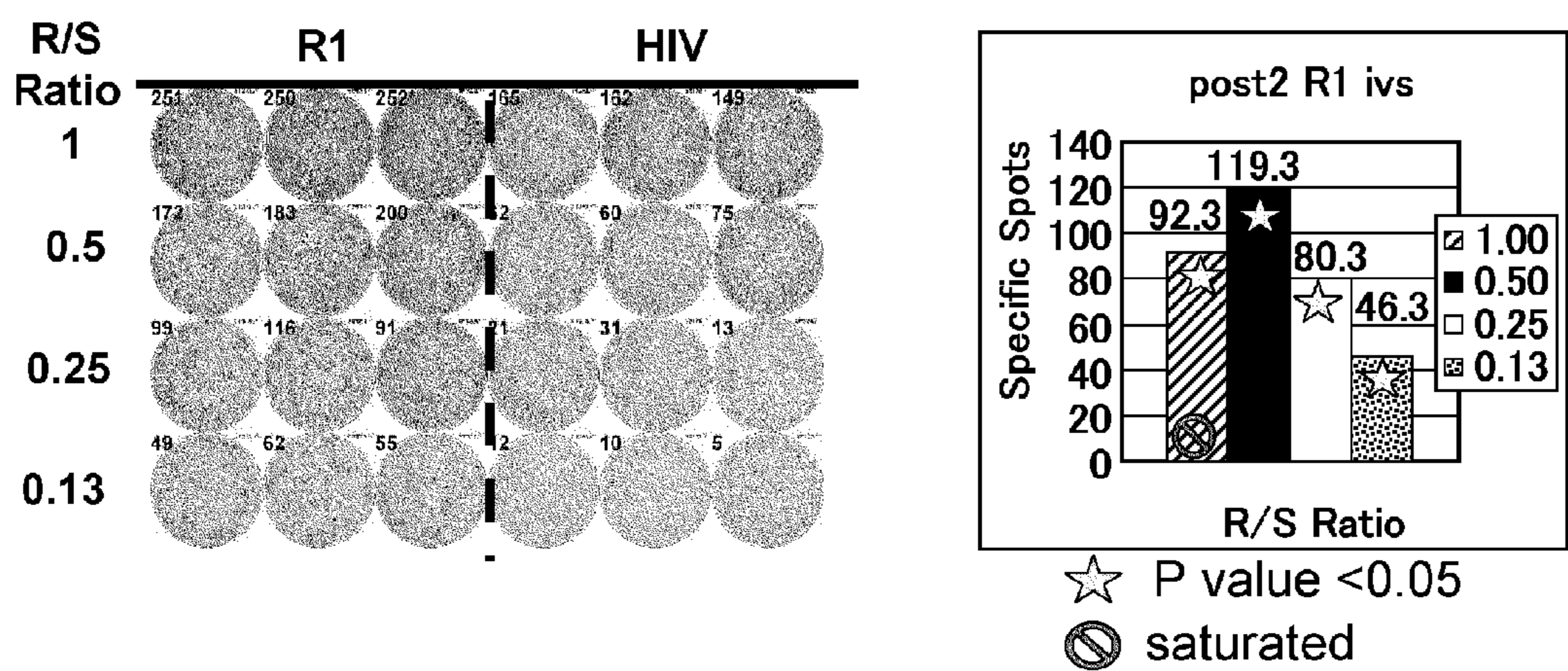


Fig. 8

a A2402-Case 1. post-2course (VEGFR1)



b A2402-Case 1. post-6course (VEGFR1)

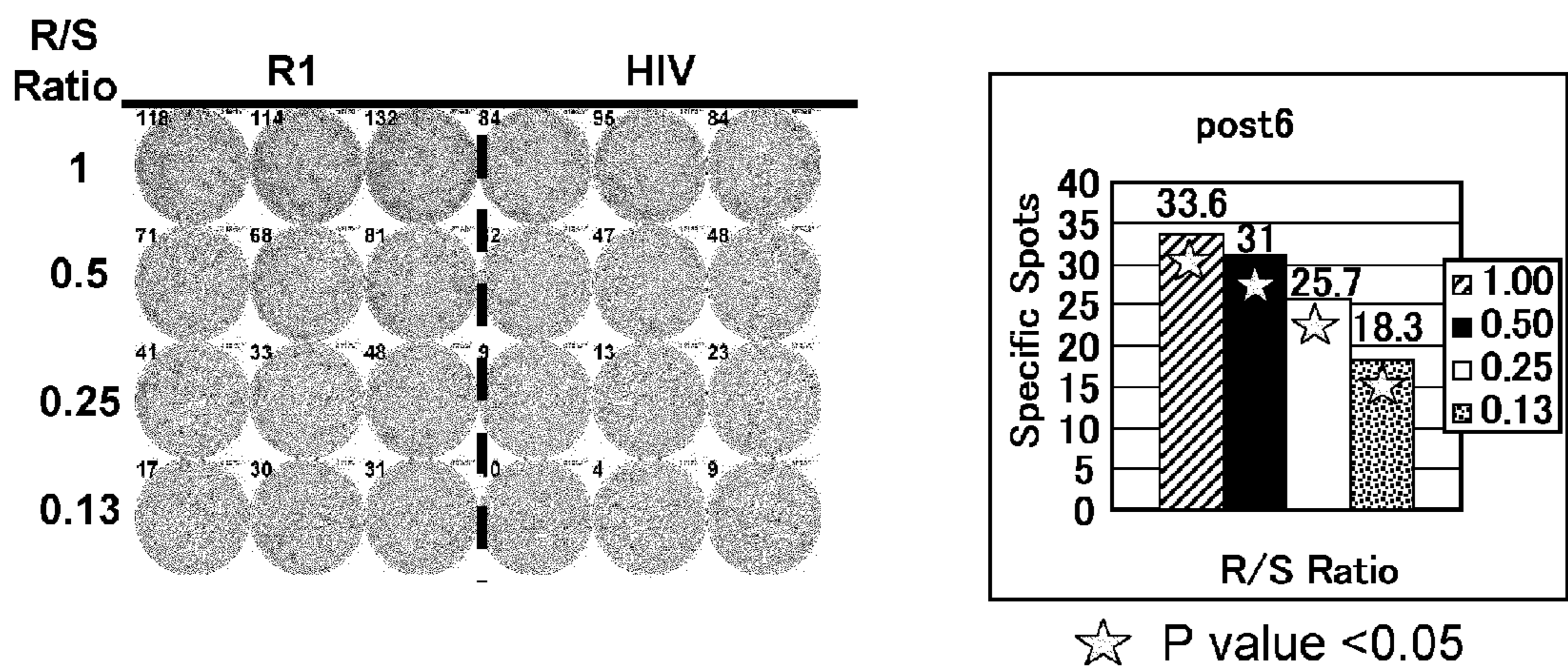
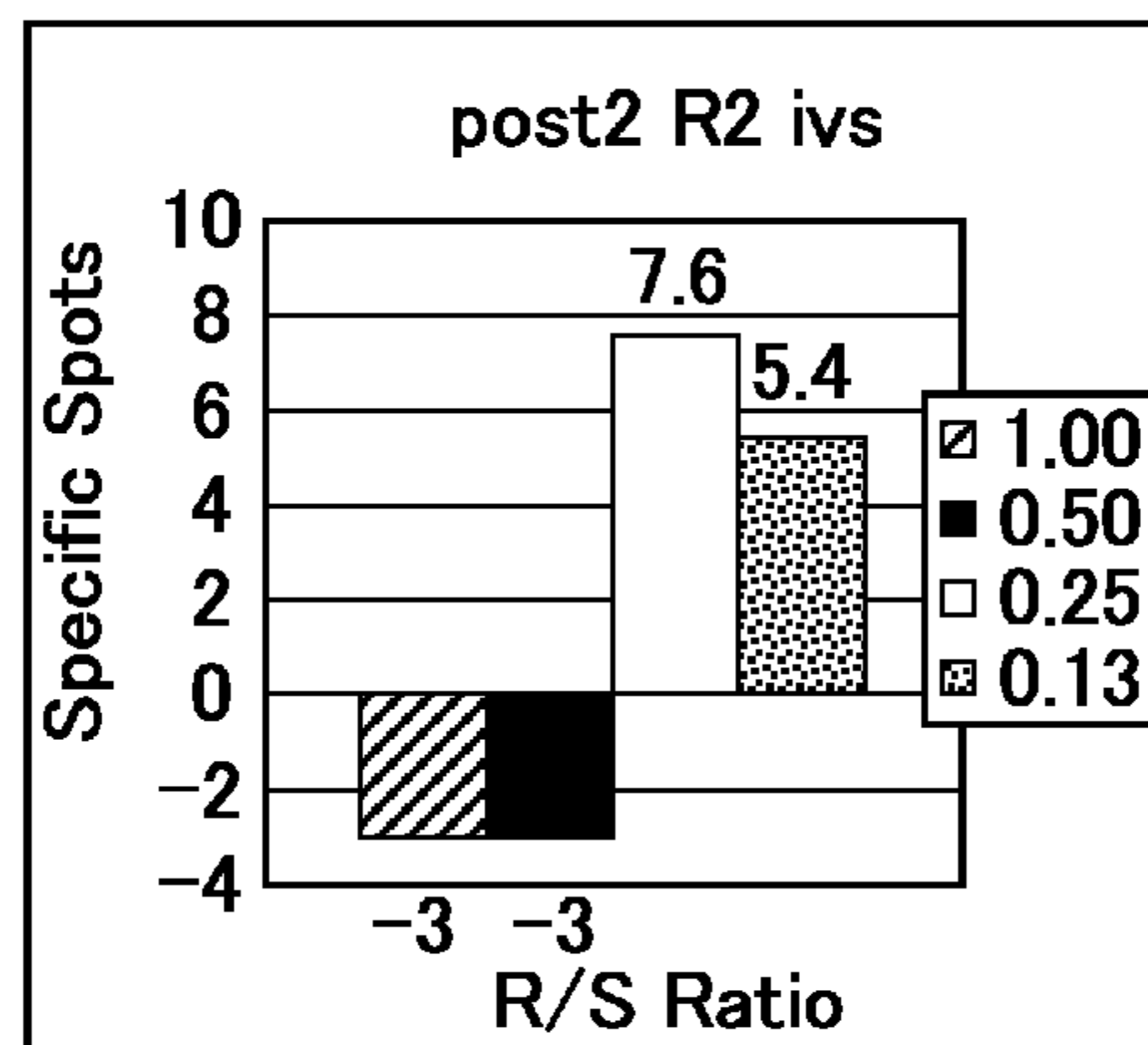


Fig. 9
a A2402-Case 1. post-2course (VEGFR2)



b A2402-Case 1. post-6course (VEGFR2)

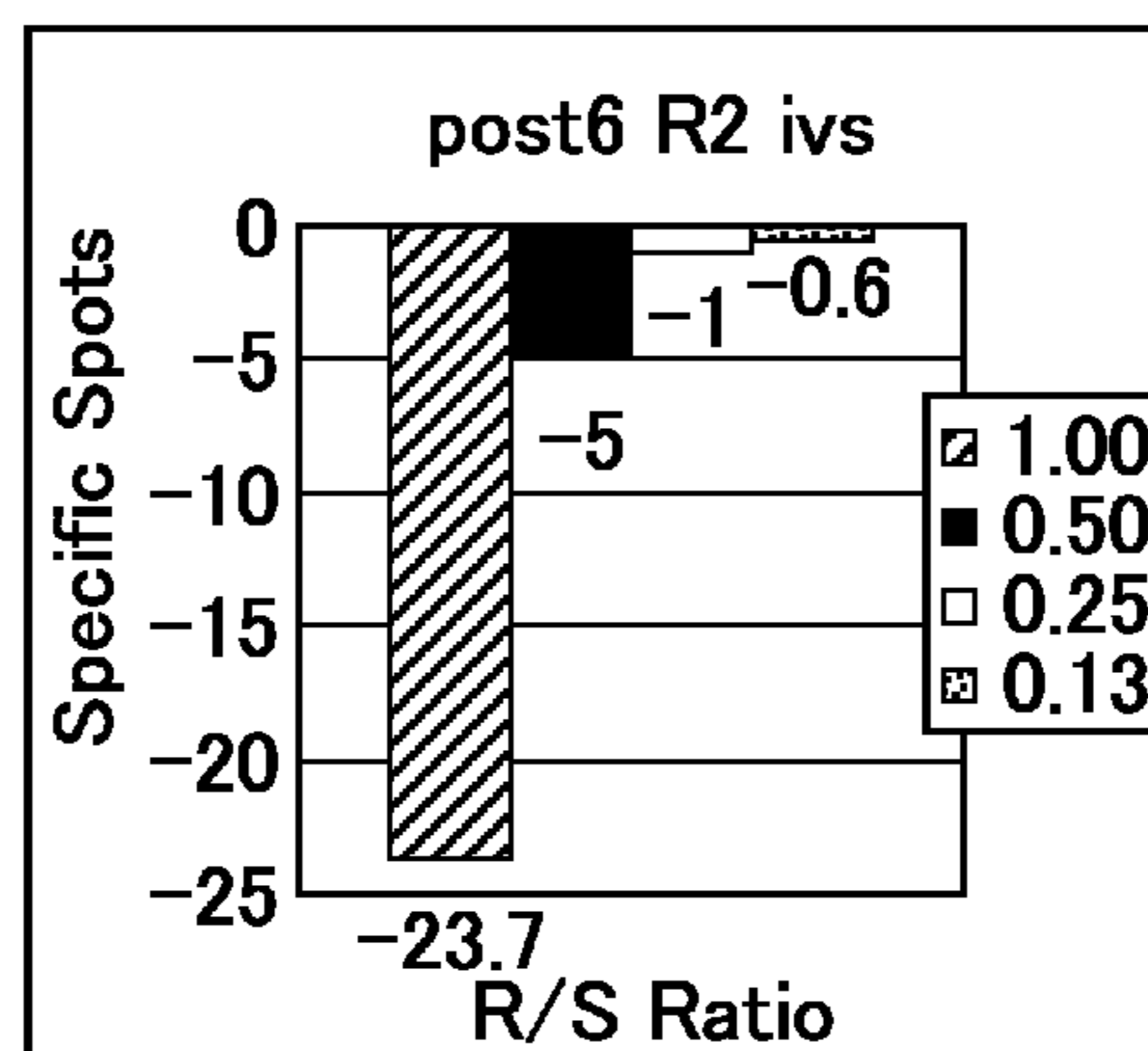
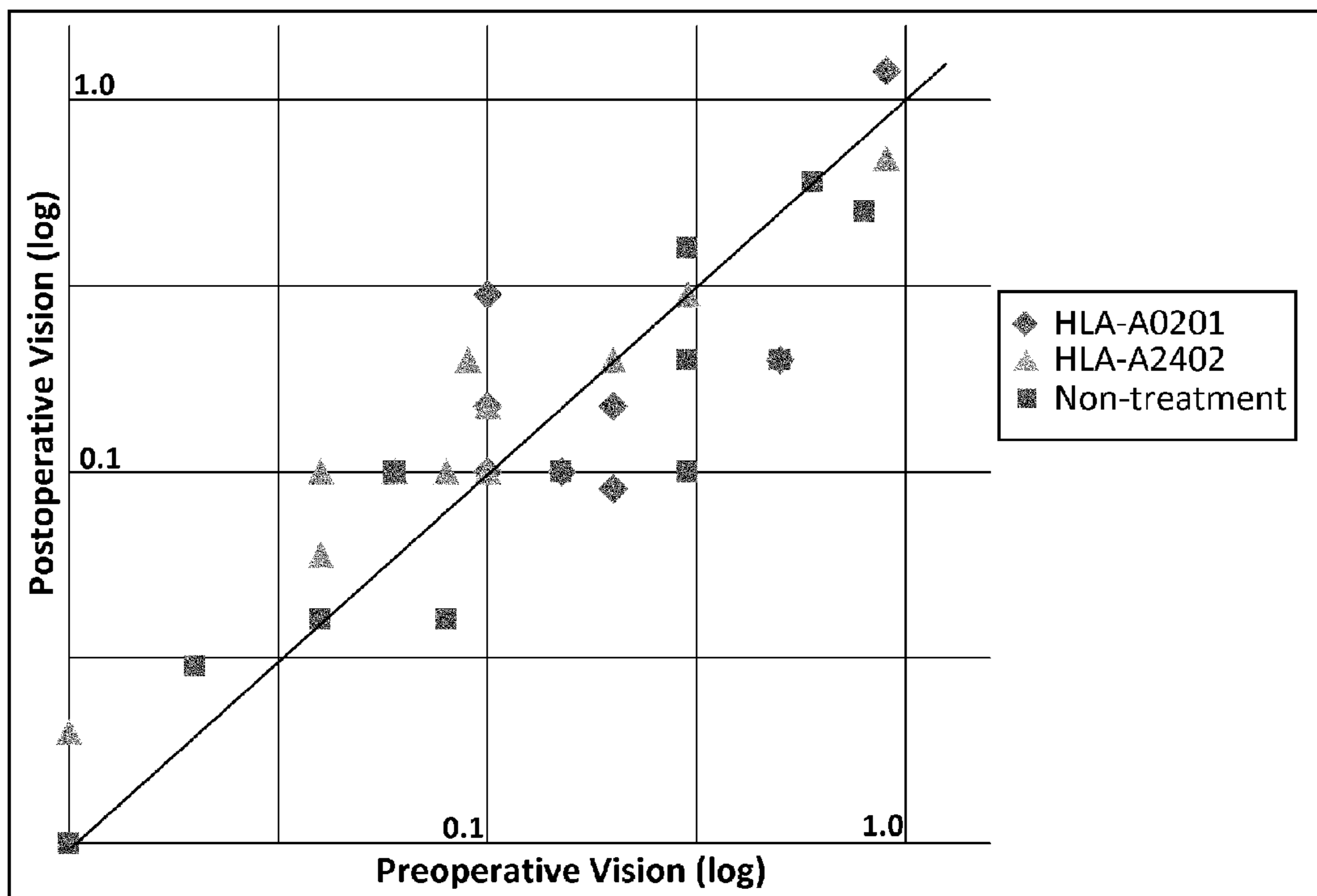


Fig. 10
Change in vision after treatment



**METHODS FOR TREATING A DISEASE
CAUSED BY CHOROIDAL
NEOVASCULARIZATION**

PRIORITY

This application is a U.S. National Phase of PCT/JP2010/003871, filed Jun. 10, 2010, which claims the benefit of Japanese Patent Application Number. 2009-140363 filed Jun. 11, 2009, the contents of which are hereby incorporated herein by reference in their entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING

This application includes a Sequence Listing as a text file named "SEQTXT 87331-027400US-825489.txt" created Dec. 7, 2011 and containing 44,327 bytes. The material contained in this text file is incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

This application claims the benefit of Japanese Patent Application Number. 2009-140363 filed Jun. 11, 2009, the contents of which are hereby incorporated herein by reference in their entirety for all purposes.

The present invention relates to pharmaceutical compositions and vaccines for treatment and/or prevention of diseases caused by neovascularization in the choroid (neovascular maculopathy). The present invention also relates to pharmaceutical compositions and vaccines for inhibiting neovascularization in the choroid.

BACKGROUND ART

Exudative age-related macular degeneration (AMD) caused by choroid neovascularization (CNV) is one of the major causes for severe visual impairment in developed countries. Evidence to date suggests that vascular endothelial growth factor (VEGF) plays a central role in the development of CNV. For example, it has been reported that CNV is suppressed by compounds that inhibit the production of VEGF or compounds that inhibit the signal transduction pathway of VEGF. Furthermore, it has also been reported that anti-VEGF antibodies show higher therapeutic efficacy compared to conventional therapeutic methods including photodynamic therapy. Therefore, in recent years, anti-VEGF agents have become a main option for drug therapy against CNV.

VEGF signaling is mediated by two types of receptor tyrosine kinases, i.e., VEGF receptor 1 (VEGFR-1) and VEGF receptor 2 (VEGFR-2). The two receptors are expressed on the human CNV membrane and the laboratory mouse CNV membrane. However, the role of VEGFR-1 signal transduction pathway in CNV is still controversial. For example, one study reports that the inhibition of VEGFR-1 signaling by oral administration of an antibody, gene knock-down, or siRNA inhibits CNV. Another study reports that in the eye, activation of VEGFR-1 by VEGF or placental growth factor 1 (PlGF1), which is a ligand of VEGFR-2, leads to activation of CNV via activation of VEGFR-2 by SPARC. On the other hand, for VEGFR-2, the finding that activation of VEGFR-2 signaling promotes CNV growth is generally accepted. Thus, antiangiogenic approaches targeting VEGFR-2, such as systemic or local administration of anti-VEGFR-2 agents or VEGFR-2 antibodies, and intravitreal administration of siRNA, are expected to inhibit VEGFR-2 signaling and CNV growth.

However, the problem with currently available anti-VEGF agents is that they need to be injected repeatedly at 4- to 6-week intervals. Furthermore, there is a high risk of severe complications such as endophthalmitis and retinal detachment. Therefore, it is desirable to establish a novel therapeutic method that replaces currently used anti-VEGF agents.

A vaccine using a peptide derived from human VEGF receptor 2 is known to induce cytotoxic T-lymphocytes (CTLs) in tumor tissues which have potent cytotoxicity against VEGFR-2-expressing endothelial cells (Patent Document 1). A vaccine using a peptide derived from human VEGF receptor 1 is also known to induce CTLs which have potent cytotoxicity against VEGFR-1-expressing endothelium (Patent Document 2). Furthermore, a vaccine using a peptide derived from VEGF receptor 2 has been confirmed to have CNV inhibitory effects in mice (Patent Document 3). However, as in other tissues, there are many unclear points in the mechanism of neovascularization in the choroid, and the presence of a vaccine that effectively inhibits CNV in human choroid is not known.

CITATION LIST

Patent Literature

[PTL 1] WO 2004/024766
[PTL 2] WO 2006/093030
[PTL 3] WO 2008/099908

SUMMARY OF INVENTION

Technical Problem

The present invention was achieved in view of the above circumstances. An objective to be achieved by the present invention is to provide novel pharmaceutical agents and methods for treating and/or preventing a disease caused by neovascularization in human choroid (neovascular maculopathy).

Solution to Problem

The present inventors administered a pharmaceutical composition/vaccine containing a VEGFR-1-derived peptide to neovascular maculopathy patients, and as a result discovered that this can effectively inhibit human CNV without causing problems suggestive of safety issue, and thereby completed the present invention.

More specifically, the present invention provides a pharmaceutical composition for treating and/or preventing a disease caused by neovascularization in human choroid (neovascular maculopathy), comprising as an active ingredient at least a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention also provides a vaccine for treating and/or preventing a disease caused by neovascularization in human choroid, comprising as an active ingredient at least a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention also provides a pharmaceutical composition for inhibiting neovascularization in human choroid, comprising as an active ingredient at least a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

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Furthermore, the present invention provides a vaccine for inhibiting neovascularization in human choroid, comprising as an active ingredient at least a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention provides a method for treating and/or preventing a disease caused by neovascularization in human choroid, comprising the step of administering to a subject at least a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention also provides a method for inhibiting neovascularization in human choroid, comprising the step of administering to a subject at least a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention further provides use of a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof in manufacturing a pharmaceutical composition for treating and/or preventing a disease caused by neovascularization in human choroid.

Furthermore, the present invention provides use of a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, in manufacturing a vaccine for treating and/or preventing a disease caused by neovascularization in human choroid.

The present invention also provides use of a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, in manufacturing a pharmaceutical composition for inhibiting neovascularization in human choroid.

In addition, the present invention provides use of a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, in manufacturing a vaccine for inhibiting neovascularization in human choroid.

The present invention further provides a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells for use in treating and/or preventing a disease caused by neovascularization in human choroid.

Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition for treating or preventing a disease caused by neovascularization in human choroid, wherein the method or process includes the step of formulating a pharmaceutically or physiologically acceptable carrier with an active ingredient of a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition for treating or preventing a disease caused by neovascularization in human choroid, wherein the method or process includes the steps of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

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Alternatively, in one embodiment, in the present invention, VEGFR-1-derived peptide may also be administered in combination with a VEGFR-2-derived peptide for treating or inhibiting human CNV. Accordingly, the present invention provides a pharmaceutical composition for treating and/or preventing a disease caused by neovascularization in human choroid (neovascular maculopathy), comprising as an active ingredient at least one type each of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention also provides a vaccine for treating and/or preventing a disease caused by neovascularization in human choroid, comprising as an active ingredient at least one type each of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention also provides a pharmaceutical composition for inhibiting neovascularization in human choroid, comprising as an active ingredient at least one type each of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

Furthermore, the present invention provides a vaccine for inhibiting neovascularization in human choroid, comprising as an active ingredient at least one type each of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention provides a method for treating and/or preventing a disease caused by neovascularization in human choroid, comprising the step of administering to a subject at least one type each of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention also provides a method for inhibiting neovascularization in human choroid, comprising the step of administering to a subject at least one type each of a peptide selected from the group consisting of;

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(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

The present invention further provides use of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells,

in manufacturing a pharmaceutical composition for treating and/or preventing a disease caused by neovascularization in human choroid.

Furthermore, the present invention provides use of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells,

in manufacturing a vaccine, and/or a polynucleotide encoding thereof for treating and/or preventing a disease caused by neovascularization in human choroid.

The present invention also provides use of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, in manufacturing a pharmaceutical composition for inhibiting neovascularization in human choroid.

In addition, the present invention provides use of a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, in manufacturing a vaccine for inhibiting neovascularization in human choroid.

In addition, the present invention provides a peptide selected from the group consisting of;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof,

for use in treating or preventing a disease caused by neovascularization in human choroid.

In addition, the present invention provides a peptide selected from the group consisting of;

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(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, for use in inhibiting neovascularization in human choroid.

Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition for treating or preventing a disease caused by neovascularization in human choroid, wherein the method or process includes the step of formulating a pharmaceutically or physiologically acceptable carrier with an active ingredient selected from among;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, as active ingredients.

Alternatively, the present invention further provides a method or process for manufacturing a vaccine for inhibiting neovascularization in human choroid, wherein the method or process includes the step of formulating a pharmaceutically or physiologically acceptable carrier with an active ingredient selected from among;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof, as active ingredients.

In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition for treating or preventing a disease caused by neovascularization in human choroid, wherein the method or process includes the steps of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is selected from among;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

In another embodiment, the present invention also provides a method or process for manufacturing a vaccine for inhibiting neovascularization in human choroid, wherein the method or process includes the steps of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is selected from among;

(a) a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof and

(b) a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells, and/or a polynucleotide encoding thereof.

More, specifically, the present invention provides the following [1] to [30];

[1] A pharmaceutical composition for treating and/or preventing a disease caused by neovascularization in human choroid (neovascular maculopathy), comprising as an active ingredient at least one type of the peptides of (a) peptides comprising an amino acid sequence derived from a VEGF receptor 1 protein and having an activity of inducing cytotoxic T cells, or a polynucleotide encoding thereof,

[2] The pharmaceutical composition of [1], wherein the above-mentioned peptides of (a) include the peptide of (i) and (ii) below:

(i) at least one peptide comprising any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4;

(ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4,

[3] The pharmaceutical composition of [2], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:

(1) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine;

(2) a peptide in which the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;

(3) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine, and the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;

(4) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan;

(5) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and

(6) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,

[4] The pharmaceutical composition of any one of [1] to [3], wherein the composition further comprises at least one type of the peptides of (b) peptides comprising an amino acid sequence derived from a VEGF receptor 2 protein and having an activity of inducing cytotoxic T cells,

[5] The pharmaceutical composition of [4], wherein the above-mentioned peptides of (b) include (i) and (ii) below:

(i) at least one peptide comprising any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 to 17; and

(ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 to 17,

[6] The pharmaceutical composition of [5], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:

(1) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine;

(2) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;

(3) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;

(4) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan;

(5) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and

(6) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,

[7] The pharmaceutical composition of any one of [1] to [6], wherein the disease caused by neovascularization in the choroid (neovascular maculopathy) is selected from exudative age-related macular degeneration, myopic macular degeneration, angioid streaks, central exudative chorioretinopathy, various retinal pigment epitheliopathy, choroidal atrophy, choroideremia, and choroidal osteoma,

[8] The pharmaceutical composition of any one of [1] to [7], which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24,

[9] A vaccine for treating and/or preventing a disease caused by neovascularization in human choroid (neovascular maculopathy), comprising as an active ingredient at least one type of the peptides of (a) peptides comprising an amino acid sequence derived from a VEGF receptor 1 protein and having an activity of inducing cytotoxic T cells, or a polynucleotide encoding thereof,

[10] The vaccine of [9], wherein the above-mentioned peptides of (a) include the peptide of (i) and (ii) below:

(i) at least one peptide comprising any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4; and

(ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4,

[11] The vaccine of [10], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:

(1) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine;

(2) a peptide in which the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;

(3) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine, and the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;

(4) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan;

(5) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and

(6) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is

- phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,
- [12] The vaccine of any one of [9] to [11], wherein the vaccine further comprises at least one type of the peptides of (b) peptides comprising an amino acid sequence derived from a VEGF receptor 2 protein and having an activity of inducing cytotoxic T cells,
- [13] The vaccine of [12], wherein the above-mentioned peptides of (b) include (i) and (ii) below:
- (i) at least one peptide comprising the amino acid sequence of any one of SEQ ID NOs: 5 to 17; and
- (ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 to 17,
- [14] The vaccine of [13], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:
- (1) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine;
- (2) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;
- (3) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;
- (4) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan;
- (5) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and
- (6) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,
- [15] The vaccine of any one of [9] to [14], wherein the disease caused by neovascularization in the choroid (neovascular maculopathy) is selected from exudative age-related macular degeneration, myopic macular degeneration, angioid streaks, central exudative chorioretinopathy, various retinal pigment epitheliopathy, choroidal atrophy, choroideremia, and choroidal osteoma,
- [16] The vaccine of any one of [9] to [15], which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24,
- [17] A pharmaceutical composition for inhibiting neovascularization in human choroid, comprising as an active ingredient at least one type of the peptides of (a) peptides comprising an amino acid sequence derived from a VEGF receptor 1 protein and having an activity of inducing cytotoxic T cells, or a polynucleotide encoding thereof,
- [18] The pharmaceutical composition of [17], wherein the above-mentioned peptides of (a) include the peptide of (i) and (ii) below:
- (i) at least one peptide comprising any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4; and

- (ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4,
- [19] The pharmaceutical composition of [18], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:
- (1) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine;
- (2) a peptide in which the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;
- (3) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine, and the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;
- (4) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan;
- (5) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and
- (6) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,
- [20] The pharmaceutical composition of any one of [17] to [19], wherein the composition further comprises at least one type of the peptides of (b) peptides comprising an amino acid sequence derived from a VEGF receptor 2 protein and having an activity of inducing cytotoxic T cells,
- [21] The pharmaceutical composition of [20], wherein the above-mentioned peptides of (b) include (i) and (ii) below:
- (i) at least one peptide comprising the amino acid sequence of any one of SEQ ID NOs: 5 to 17; and
- (ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 to 17,
- [22] The pharmaceutical composition of [21], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:
- (1) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine;
- (2) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;
- (3) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;
- (4) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan;
- (5) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and

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- (6) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,
- [23] The pharmaceutical composition of any one of [15] to [19], which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24,
- [24] A vaccine for inhibiting neovascularization in human choroid, comprising as an active ingredient at least one type of the peptides of (a) peptides comprising an amino acid sequence derived from a VEGF receptor 1 protein and having an activity of inducing cytotoxic T cells, or a polynucleotide encoding thereof,
- [25] The vaccine of [24], wherein the above-mentioned peptides of (a) include the peptide of (i) and (ii) below:
- (i) at least one peptide comprising any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4; and
- (ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from group consisting of SEQ ID NOs: 1 to 4,
- [26] The vaccine of [25], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:
- (1) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine;
- (2) a peptide in which the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;
- (3) a peptide in which the second amino acid from the N terminus of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is leucine or methionine, and the C-terminal amino acid of any one of amino acid sequence of SEQ ID NOs: 1 to 3 is valine or leucine;
- (4) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan;
- (5) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and
- (6) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is phenylalanine, leucine, isoleucine, tryptophan, or methionine,
- [27] The vaccine of any one of [24] to [26], wherein the vaccine further comprises at least one type of the peptides of (b) peptides comprising an amino acid sequence derived from a VEGF receptor 2 protein and having an activity of inducing cytotoxic T cells,
- [28] The vaccine of [27], wherein the above-mentioned peptides of (b) include the peptide of (i) and (ii) below:
- (i) at least one peptide comprising the amino acid sequence of any one of SEQ ID NOs: 5 to 17; and
- (ii) at least one peptide comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in any one of the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 to 17,
- [29] The vaccine of [28], wherein the above-mentioned peptide of (ii) is any one of peptides of (1) to (6) below:

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- (1) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine;
- (2) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;
- (3) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is leucine or methionine and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 11 to 17 is valine or leucine;
- (4) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan;
- (5) a peptide in which the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine; and
- (6) a peptide in which the second amino acid from the N terminus of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, tyrosine, methionine, or tryptophan and the C-terminal amino acid of any one of the amino acid sequence of SEQ ID NOs: 5 to 10 is phenylalanine, leucine, isoleucine, tryptophan, or methionine, and
- [30] The vaccine of any one of [24] to [29], which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24.

Advantageous Effects of Invention

The present invention can provide pharmaceutical compositions and vaccines effective for treating and preventing diseases caused by neovascularization in human choroid (neovascular maculopathy). Furthermore, the present invention can provide pharmaceutical compositions and vaccines effective for inhibiting neovascularization in human choroid.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1A-1C show the symptomatic relief of an age-related macular degeneration patient HLA-A0201-Case 1 who has been given a VEGFR-1-derived peptide and a VEGFR-2-derived peptide. (A) shows the tomographic images before starting the administration, (B) shows ocular fundus photographs before starting the administration, (C) shows fluorescein fundus photography before starting the administration. The arrows of (A) and (D) indicate the line of the pigment epithelium and of (B) and (E) indicate the detachment of the pigment epithelium.

FIG. 1D-1F show the symptomatic relief of an age-related macular degeneration patient HLA-A0201-Case 1 who has been given a VEGFR-1-derived peptide and a VEGFR-2-derived peptide. (D) shows the tomographic images five months after starting the administration, (E) shows the ocular fundus photograph five months after starting the administration, (F) shows fluorescein fundus photography five months after starting the administration. The arrows of (A) and (D) indicate the line of the pigment epithelium and of (B) and (E) indicate the detachment of the pigment epithelium.

FIG. 2A-2B show retinal tomographic images acquired by optical coherence tomography performed on a single case of an age-related macular degeneration patient HLA-A0201-Case3 who has been given a VEGFR-1-derived peptide and a VEGFR-2-derived peptide. (A) shows the tomographic images before starting the administration and (B) shows the tomographic images one month after starting the administration.

tion. The arrows indicate edema, and the dashed arrow indicates an apparently a fibrosed and hypoactive neovascular membrane.

FIG. 3 shows the symptomatic relief and the recovery of vision of an age-related macular degeneration patient HLA-A2402-Case 1 who has been given a VEGFR-1-derived peptide and a VEGFR-2-derived peptide. Upper photographs show ocular fundus photographs and lower photographs show retinal tomographic images subretinal hemorrhages (arrowhead) disappeared and the vision was improved (parenthetic value) after starting the treatment. Additionally, the anatomy of macular was remaining the same.

FIG. 4a-4b show the VEGFR1 peptide-specific response of HLA-A0201-Case1. The PBMCs of pre-treatment (a), and post-1 course (b) were tested. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (lower left panel) and VEGFR1 peptide-specific spots (lower right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). Circular mark indicates that spot counts are saturated.

FIG. 4c-4d show the VEGFR1 peptide-specific response of HLA-A0201-Case1. The PBMCs of post-2 courses (c), and post-3 courses (d) were tested. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (lower left panel) and VEGFR1 peptide-specific spots (lower right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). Circular mark indicates that spot counts are saturated.

FIG. 4e shows the VEGFR1 peptide-specific response of HLA-A0201-Case1. The PBMCs of post-4 courses (e) was tested. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (lower left panel) and VEGFR1 peptide-specific spots (lower right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). Circular mark indicates that spot counts are saturated.

FIG. 5a-5b show the VEGFR2 peptide-specific response of HLA-A0201-Case1. The PBMCs of pre-treatment (a), and post-1 course (b) were tested. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR2-A2-773 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (lower left panel) and VEGFR2 peptide-specific spots (lower right panel) is indicated in the graphs.

FIG. 5c-5d show the VEGFR2 peptide-specific response of HLA-A0201-Case1. The PBMCs of post-2 courses (c), and post-3 courses (d) were tested. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR2-A2-773 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (lower left panel) and VEGFR2 peptide-specific spots (lower right panel) is indicated in the graphs.

FIG. 5e shows the VEGFR2 peptide-specific response of HLA-A0201-Case1. The PBMCs of post-4 courses (e) was tested. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR2-A2-773 pep-

tide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (lower left panel) and VEGFR2 peptide-specific spots (lower right panel) is indicated in the graphs.

FIG. 6a shows the VEGFR1 peptide-specific response of HLA-A0201-Case3. The responses of the PBMCs of post-1 course (a) is shown as representative results. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (middle left panel) and VEGFR1 peptide-specific spots (middle right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). The VEGFR1 peptide-specific T cell receptor was detected by HLA-A*0201/VEGFR1 dextramer (lower panel).

FIG. 6b shows the VEGFR1 peptide-specific response of HLA-A0201-Case3. The responses of the PBMCs of post-3 courses (b) is shown as representative results. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (middle left panel) and VEGFR1 peptide-specific spots (middle right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). The VEGFR1 peptide-specific T cell receptor was detected by HLA-A*0201/VEGFR1 dextramer (lower panel).

FIG. 6c shows the VEGFR1 peptide-specific response of HLA-A0201-Case3. The responses of the PBMCs of post-4 courses (c) is shown as representative results. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (middle left panel) and VEGFR1 peptide-specific spots (middle right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). The VEGFR1 peptide-specific T cell receptor was detected by HLA-A*0201/VEGFR1 dextramer (lower panel).

FIG. 6d shows the VEGFR1 peptide-specific response of HLA-A0201-Case3. The responses of the PBMCs of post-5 courses (d) is shown as representative results. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A2-770 peptide (upper left panel) or HIV-Env peptide (upper right panel) is shown. R/S; responder/stimulator ratio. The number of spot counts (middle left panel) and VEGFR1 peptide-specific spots (middle right panel) is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; $P < 0.05$). The VEGFR1 peptide-specific T cell receptor was detected by HLA-A*0201/VEGFR1 dextramer (lower panel).

FIG. 7a-7c show the VEGFR2 peptide-specific response of HLA-A0201-Case3. The PBMCs of pre-treatment (a), post-1 course (b), and post-3 courses (c) were tested. In each figure, the number of spot counts against VEGFR2-A2-773 peptide-pulsed TISI or HIV-Env peptide-pulsed TISI (left panel) and VEGFR2 peptide-specific spots (right panel) is indicated in the graphs.

FIG. 7d-7e show the VEGFR2 peptide-specific response of HLA-A0201-Case3. The PBMCs of post-4 courses (d) and post-5 courses (e) were tested. In each figure, the number of spot counts against VEGFR2-A2-773 peptide-pulsed TISI or

HIV-Env peptide-pulsed TISI (left panel) and VEGFR2 peptide-specific spots (right panel) is indicated in the graphs.

FIG. 8a-8b show the VEGFR1 peptide-specific response of HLA-A2402-Cas1. The responses of the PBMCs of post-2 course (a) and post-6 courses (b) are shown as representative results. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR1-A24-1084 peptide (left panel) or HIV-Env peptide (right panel) is shown. R/S; responder/stimulator ratio. The number of VEGFR1 peptide-specific spots is indicated in the graphs. Statistical analysis was performed using unpaired Student's t-test (Star mark; P<0.05). Circular mark indicates that spot counts are saturated.

FIG. 9a-9b show the VEGFR2 peptide-specific response of HLA-A2402-Cas1. The responses of the PBMCs of post-2 course (a) and post-6 courses (b) are shown as representative results. In each figure, the photograph of ELISPOT plate in which the PBMCs were stimulated by VEGFR2-A24-169 peptide (left panel) or HIV-Env peptide (right panel) is shown. R/S; responder/stimulator ratio. The number of VEGFR2 peptide-specific spots was indicated in the graphs.

FIG. 10 shows the changes in vision of subjects after treatment. The visions of treatment groups were improved compared to non-treatment group with significant difference (p value=0.015).

DESCRIPTION OF EMBODIMENTS

Definitions

The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue(s) may be modified residue(s), or non-naturally occurring residue(s), such as artificial chemical mimetic(s) of corresponding naturally occurring amino acid(s), as well as to naturally occurring amino acid polymers.

The term "amino acid" as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that similarly function to the naturally occurring amino acids. Amino acid may be either L-amino acids or D-amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those modified after translation in cells (e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine). The phrase "amino acid analog" refers to compounds that have the same basic chemical structure (an alpha carbon bound to a hydrogen, a carboxy group, an amino group, and an R group) as a naturally occurring amino acid but have one or more modified R group(s) or modified backbones (e.g., homoserine, norleucine, methionine, sulfoxide, methionine methyl sulfonium). The phrase "amino acid mimetic" refers to chemical compounds that have different structures but similar functions to general amino acids.

Amino acids may be referred to herein by their commonly known three letter symbols or the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

The terms "gene", "polynucleotides", "nucleotides" and "nucleic acids" are used interchangeably herein and, unless otherwise specifically indicated are referred to by their commonly accepted single-letter codes.

The term "composition" as used herein is intended to encompass a product including the specified ingredients in

the specified amounts, as well as any product that results, directly or indirectly, from combination of the specified ingredients in the specified amounts. Such term in relation to "pharmaceutical composition", is intended to encompass a product including the active ingredient(s), and any inert ingredient(s) that make up the carrier, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, in the context of the present invention, the phrase "pharmaceutical composition" encompasses any composition made by admixing a compound of the present invention and a pharmaceutically or physiologically acceptable carrier. The phrase "pharmaceutically acceptable carrier" or "physiologically acceptable carrier", as used herein, means a pharmaceutically or physiologically acceptable material, composition, substance or vehicle, including but not limited to, a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the active ingredient(s) from one organ, or portion of the body, to another organ, or portion of the body.

Unless otherwise defined, the terms "cytotoxic T lymphocyte", "cytotoxic T cell" and "CTL" are used interchangeably herein and unless otherwise specifically indicated, refer to a sub-group of T lymphocytes that are capable of recognizing non-self cells (e.g., virus-infected cells) and inducing the death of such cells.

Unless otherwise defined, the terms "HLA-A24" refers to the HLA-A24 type containing the subtypes such as HLA-A*2402.

Unless otherwise defined, the term "HLA-A02", as used herein, representatively refers to the subtypes such as HLA-A*0201.

Unless otherwise defined, the term "kit" as used herein, is used in reference to a combination of reagents and other materials. It is not intended that the term "kit" be limited to a particular combination of agents and/or materials.

To the extent that the methods and compositions of the present invention find utility in the context of the "treatment" of disease caused by neovascularization in human choroid (neovascular maculopathy), a treatment is deemed "efficacious" if it leads to clinical benefit such as, decrease in the detachment of pigment epithelium, amelioration of the detachment of pigment epithelium, reduced leakage, or amelioration of distortion in the subject. Efficaciousness is determined in association with any known method for treating the disease caused by neovascularization in human choroid (neovascular maculopathy).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present invention belongs.

The present invention relates to pharmaceutical compositions for treating and/or preventing a disease caused by neovascularization in the choroid (neovascular maculopathy) and pharmaceutical compositions for inhibiting neovascularization in the choroid, which comprise as an active ingredient a peptide comprising an amino acid sequence derived from a VEGFR-1 protein and having an activity of inducing cytotoxic T cells (hereinafter referred to as "VEGFR-1 peptide") (hereinafter, the composition may together be referred to as "pharmaceutical composition of the present invention") and/or a polynucleotide encoding thereof. Furthermore, the present invention relates to vaccines for treating and/or preventing a disease caused by neovascularization in the choroid (neovascular maculopathy), and vaccines for inhibiting

neovascularization in the choroid, which comprise VEGFR-1 (hereinafter, the vaccine may together be referred to as “vaccine of the present invention”) and/or a polynucleotide encoding thereof. The pharmaceutical composition and vaccine above can comprise any other substances, for example immune stimulators. Preferably, a peptide comprising an amino acid sequence derived from other protein and having an activity of inducing cytotoxic T cells can be comprised. More preferably, a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells (hereinafter referred to as “VEGFR-2 peptide”). The present invention is based on the present inventors’ finding that pharmaceutical compositions/vaccines comprising VEGFR-1 peptide are effective for inhibiting neovascularization in the choroid.

VEGFR-1 Peptide

The VEGFR-1 peptide contained in the pharmaceutical compositions and vaccines of the present invention (hereinafter, “VEGFR-1 peptide”) may be referred to as “peptide of the present invention”) can be obtained by synthesizing peptides from any position in the amino acid sequence of a known VEGFR-1 protein. The present invention can contain VEGFR-2 peptide and also can be obtained by synthesizing peptides from any position in the amino acid sequence of a known VEGFR-2. Amino acid sequences of human VEGFR-1 and human VEGFR-2 are known, and those skilled in the art can easily obtain them from protein sequence databases and such available on the Internet. An example of the amino acid sequence of a human VEGFR-1 protein is the amino acid sequence of SEQ ID NO: 19 (the amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. NM_002019). An example of the amino acid sequence of a human VEGFR-2 protein is the amino acid sequence of SEQ ID NO: 21 (the amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. NM_002253).

Peptide synthesis can be performed according to methods conventionally used in peptide chemistry. Conventional synthesis methods are described in documents such as “Peptide Synthesis”, Interscience, New York, 1966; “The Proteins”, Vol. 2, Academic Press Inc., New York, 1976; “Peptide Synthesis (Peptide Gosei)”, Maruzen, 1975; “Fundamentals and Experiments of Peptide Synthesis (Peptide Gosei no Kiso to Jikken)”, Maruzen, 1985; and “The sequel of Development of Pharmaceuticals (Zoku Iyakuin no Kaihatsu)”, Vol. 14, Peptide Synthesis (Peptide Gosei), Hirokawa Shoten, 1991, and in publications such as International Publication No. WO 99/67288. Peptides of the present invention may also be synthesized by known genetic engineering methods. The following is an example of a genetic engineering synthesis method. A vector into which a DNA encoding a peptide of the present invention has been inserted is introduced into suitable host cells to produce transformed cells. The peptides of the present invention can be obtained by collecting the peptides produced in these transformed cells. The peptides of the present invention may also be produced initially as a fusion protein, which is then cleaved using an appropriate protease to obtain the peptides.

In a method for preparing a fusion protein, a polynucleotide encoding a peptide of the present invention may be ligated in frame with a polynucleotide encoding another peptide, and this may be inserted into an expression vector for expression in a host. Techniques known to those skilled in the art can be used for this purpose. For peptides fused with the peptides of the present invention, one may use known peptides such as FLAG (Hopp, T. P. et al., *BioTechnology* (1988) 6, 1204-1210), 6× His consisting of six histidine (His) resi-

dues, 10× His, influenza hemagglutinin (HA), human c-myc fragments, VSV-GP fragments, p 18HIV fragments, T7-tag, HSV-tag, E-tag, SV40T antigen fragments, lck tag, alpha-tubulin fragments, B-tag, and Protein C fragments. It is also possible to ligate the peptides of the present invention to glutathione-S-transferase (GST), influenza hemagglutinin (HA), immunoglobulin constant regions, beta-galactosidase, maltose-binding protein (MBP), or such to produce the fusion proteins. The peptides of the present invention can be obtained by treating the fusion proteins produced in this manner with a suitable protease, and then collecting the peptides of interest. The peptides can be collected by methods known to those skilled in the art, such as affinity chromatography.

As an amino acid sequence of a peptide of the present invention, for example, any sequence can be selected from the whole amino acid sequence of a VEGFR-1 protein or the whole amino acid sequence of a VEGFR-2 protein using binding affinity to HLA antigens as an indicator. Binding affinity to HLA antigens can be measured by isolating cells having HLA antigens on the cell surface, such as dendritic cells, and measuring binding of the peptides to the cells using commonly performed methods. Alternatively, binding affinity can be calculated in silico by software recently available on the Internet, such as those described in Parker K. C., *J. Immunol.* 152, 1994. When applied to the Japanese, for example, A-24 type, A-02 type, or such, which are said to be frequently expressed in the Japanese population, is preferably used as an HLA antigen to obtain effective results. HLA antigens such as the A-02 and A-24 types each include subtypes such as A-0201 or A-2402. Examples of VEGFR-1 peptides having high binding affinity to HLA-A*0201 include peptides comprising the amino acid sequences of SEQ ID NOs: 1 to 3, and examples of VEGFR-1 peptides having high binding affinity to HLA-A*2402 include peptides comprising the amino acid sequence of SEQ ID NO: 4 (WO 2006/093030). Examples of VEGFR-2 peptides having high binding affinity to HLA-A*0201 include peptides comprising the amino acid sequences of SEQ ID NOs: 11 to 17, and examples of VEGFR-2 peptides having high binding affinity to HLA-A*2402 include peptides comprising the amino acid sequences of SEQ ID NOs: 5 to 10 (WO 2004/024766). In clinical practice, peptides having high binding affinity to an HLA antigen carried by a patient requiring treatment can be suitably selected by investigating the type of the HLA antigen in advance.

Peptides having high binding affinity to an HLA antigen are highly likely to be effective as peptides having an activity to induce cytotoxic T cells (CTLs). Still, it is desirable to examine whether or not the candidate peptide selected using the presence of high binding affinity as an indicator actually has an activity to induce CTLs. The CTL-inducing activity can be confirmed by stimulating antigen-presenting cells comprising human MHC antigens (such as B-lymphocytes, macrophages, and dendritic cells), preferably dendritic cells derived from human peripheral blood mononuclear cells, with the candidate peptide; mixing the cells with CD8-positive cells; and then measuring cytotoxicity against the target cells. As the reaction system, transgenic animals produced to express a human HLA antigen (for example, those described in *Hum. Immunol.* 2000 August; 61(8):764-79 Related Articles, Books, Linkout Induction of CTL response by a minimal epitope vaccine in HLA A*0201/DR1 transgenic mice: dependence on HLA class II restricted T(H) response., Ben Mohamed L., Krishnan R., Longmate J., Auge C., Low L., Primus J., Diamond D J.) may be used. Cytotoxicity can be calculated from the radioactivity released from target cells which are radiolabeled with, for example, ⁵¹Cr or such. Alter-

natively, the activity can be examined by measuring the IFN-gamma produced and released by CTLs in the presence of antigen-presenting cells that carry peptides, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

The length of the peptides of the present invention is not particularly limited as long as they have CTL-inducing activity, but is preferably 50 amino acids or less, more preferably 30 amino acids or less, and even more preferably 15 amino acids or less. For example, when presented on antigen-presenting cells in vivo, various proteins are degraded to 9-mer peptides (nonapeptides) and are then presented. Therefore, the peptides of the present invention are desirably 9-mers (nonapeptides) or 10-mers (decapeptides). Preferred VEGFR-1 peptides include peptides comprising the amino acid sequences of SEQ ID NOs: 1 to 4 (WO 2006/093030). Preferred VEGFR-2 peptides include peptides comprising the amino acid sequences of SEQ ID NOs: 5 to 17 (WO 2004/024766).

Furthermore, one, two, or several amino acids can be substituted, deleted, added, and/or inserted to the amino acid sequences of partial peptides of naturally occurring VEGFR-1 or VEGFR-2. Herein, "several" means five or less, and preferably three or less. When modifying amino acid residues, it is desirable to substitute with amino acids in which the properties of the amino acid side chains are maintained. Examples of amino acid side chain properties are: hydrophobic amino acids (A, I, L, M, F, P, W, Y, and V); hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, and T); amino acids comprising aliphatic side chains (G, A, V, L, I, and P); amino acids comprising hydroxyl group-containing side chains (S, T, and Y); amino acids comprising sulfur atom-containing side chains (C and M); amino acids comprising carboxylic acid- and amide-containing side chains (D, N, E, and Q); amino acids comprising basic side chains (R, K, and H); and amino acids comprising aromatic group-containing side chains (H, F, Y, and W) (all amino acids are represented by one-letter codes in parentheses). Amino acid substitution within each of these groups is generally called conservative substitution. A peptide comprising a modified amino acid sequence, in which one or more amino acid residues are substituted, deleted, added, and/or inserted to a certain amino acid sequence, is known to retain the biological activity of its original peptide (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA (1984) 81, 5662-6; Zoller, M. J. and Smith, M., Nucleic Acids Res. (1982) 10, 6487-500; Wang, A. et al., Science (1984) 224: 1431-3; Dalbadie-McFarland, G. et al., Proc. Natl. Acad. Sci. USA (1982) 79: 6409-13). Preferred examples of such modified VEGFR-1 peptides include peptides comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in the amino acid sequence of any one of SEQ ID NOs: 1 to 4. Preferred examples of modified VEGFR-2 peptides include peptides comprising an amino acid sequence with one or more amino acid substitutions, deletions, additions, and/or insertions in the amino acid sequence of any one of SEQ ID NOs: 5 to 17.

Furthermore, since the regularity of sequences of peptides displayed by binding to HLA antigens is already known (J. Immunol., 152:3913, 1994; Immunogenetics, 41:178, 1995; J. Immunol., 155:4307, 1994), sequences having such regularity can be selected, or modifications based on this regularity can be carried out on the peptides obtained as described above. For example, those with high HLA-24 binding affinity are known to be peptides in which the second amino acid from the peptide N terminus is phenylalanine, tyrosine, methionine, or tryptophan, and the C-terminal amino acid is pheny-

lalanine, leucine, isoleucine, tryptophan, or methionine. Therefore, for peptides to be contained in the pharmaceutical compositions or vaccines for administration to subjects carrying the HLA-24-type HLA antigen, one can select peptides in which the second amino acid from the N terminus is phenylalanine, tyrosine, methionine, or tryptophan, and/or the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine. Alternatively, the second amino acid from the N terminus of an obtained peptide can be modified to phenylalanine, tyrosine, methionine, or tryptophan, or the C-terminal amino acid can be modified to phenylalanine, leucine, isoleucine, tryptophan, or methionine. Preferred examples of such VEGFR-1 peptides include peptides in which the second amino acid from the N terminus is modified to phenylalanine, tyrosine, methionine, or tryptophan, and/or the C-terminal amino acid is modified to phenylalanine, leucine, isoleucine, tryptophan, or methionine in the amino acid sequence of SEQ ID NO: 4.

Furthermore, preferred examples of such VEGFR-2 peptides include peptides in which the second amino acid from the N terminus is modified to phenylalanine, tyrosine, methionine, or tryptophan, and/or the C-terminal amino acid is modified to phenylalanine, leucine, isoleucine, tryptophan, or methionine in the amino acid sequence of any one of SEQ ID NOs: 5 to 10. Meanwhile, those with high HLA-02 binding affinity are known to be peptides in which the second amino acid from the peptide N terminus is leucine or methionine, and the C-terminal amino acid is valine or leucine. Therefore, as the peptides to be contained in the pharmaceutical compositions or vaccines for administration to subjects carrying the HLA-02-type HLA antigen, one can select peptides in which the second amino acid from the N terminus is leucine or methionine, and/or the C-terminal amino acid is valine or leucine. Alternatively, the second amino acid from the N terminus of the obtained peptide can be modified to leucine or methionine, and the C-terminal amino acid can be modified to valine or leucine. Preferred examples of such VEGFR-1 peptides include peptides in which the second amino acid from the N terminus is modified to leucine or methionine and/or the C-terminal amino acid is modified to valine or leucine in the amino acid sequence of any one of SEQ ID NOs: 1 to 3. An example of modified VEGFR-2 peptides for the HLA-02 type is a peptide comprising the amino acid sequence of SEQ ID NO: 11-17.

Peptides of the present invention can be obtained as described above, but when a peptide sequence is identical to a portion of the amino acid sequence of an endogenous or exogenous protein with a different function, it may cause side effects such as autoimmune diseases or allergic symptoms against specific substances. Therefore, it is preferable to use available databases to carry out homology searches, and examine whether the sequence of the obtained peptide matches the amino acid sequence of other proteins. If the peptide sequence matches the amino acid sequence of another protein, selection of that peptide sequence should preferably be avoided. If homology search shows that no peptides differing in one or two amino acids exist, the above-mentioned amino acid sequence modifications for increasing the binding affinity to HLA antigens and/or the CTL-inducing activity would not cause those problems.

Polynucleotides

The present invention also provides polynucleotides which encode any of the afore-mentioned peptides of the present invention. These include polynucleotides derived from the natural occurring VEGFR-1 gene (GenBank Accession No. NM_002019 (for example, SEQ ID NO: 18)), or VEGFR-2 gene (GenBank Accession No. NM_002253 (for example,

SEQ ID NO: 20)) as well as those having a conservatively modified nucleotide sequences thereof. Herein, the phrase “conservatively modified nucleotide sequence” refers to sequences which encode identical or essentially identical amino acid sequences. Due to the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG, and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon may be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a peptide also describes every possible silent variation of the nucleic acid. One of skill in the art will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) may be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a peptide is implicitly described in each disclosed sequence.

The polynucleotide of the present invention may be composed of DNA, RNA, or derivatives thereof. As is well known in the art, a DNA molecule is composed of bases such as the naturally occurring bases A, T, C, and G, and T is replaced by U in an RNA. One of skill will recognize that non-naturally occurring bases be included in polynucleotides, as well.

The polynucleotide of the present invention may encode multiple peptides of the present invention with or without intervening amino acid sequences. For example, the intervening amino acid sequence may provide a cleavage site (e.g., enzyme recognition sequence) of the polynucleotide or the translated peptides. Furthermore, the polynucleotide may include any additional sequences to the coding sequence encoding the peptide of the present invention. For example, the polynucleotide may be a recombinant polynucleotide that includes regulatory sequences required for the expression of the peptide or may be an expression vector (plasmid) with marker genes and such. In general, such recombinant polynucleotides may be prepared by the manipulation of polynucleotides through conventional recombinant techniques using, for example, polymerases and endonucleases.

Both recombinant and chemical synthesis techniques may be used to produce the polynucleotides of the present invention. For example, a polynucleotide may be produced by insertion into an appropriate vector, which may be expressed when transfected into a competent cell. Alternatively, a polynucleotide may be amplified using PCR techniques or expression in suitable hosts (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1989). Alternatively, a polynucleotide may be synthesized using the solid phase techniques, as described in Beaucage S L & Iyer R P, *Tetrahedron* 1992, 48: 2223-311; Matthes et al., *EMBO J* 1984, 3: 801-5.

Pharmaceutical Compositions and Vaccines Comprising VEGFR-1 Peptide and/or a Polynucleotide Encoding Thereof

The present invention provides pharmaceutical compositions for treating and/or preventing a disease caused by neovascularization in human choroid, comprising at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof as an active ingredient.

Treatment in the present invention refers to reducing symptoms characteristic of diseases caused by neovascularization in the choroid in patients who have actually developed the symptoms. In the present invention, the degree of reduction is not particularly limited, and as long as the symptoms can be

reduced, even if the degree is very slight, it is included in the meaning of the treatment of the present invention. In the present invention, prevention means suppressing in advance the progress of symptoms characteristic of diseases caused by neovascularization in the choroid. In the present invention, the degree of suppression of the progress is not limited in any way, and as long as the progress can be suppressed, even if the degree is very slight, it is included in the meaning of prevention of the present invention. The symptoms of a disease caused by neovascularization in the choroid include reduced vision. Assessment of whether or not this symptom has been reduced can be determined by a vision test. Furthermore, one can determine whether or not the progress of symptoms is suppressed by evaluating the activity of choroidal neovessels through examinations using fluorescein fundus photography or optical coherence tomography.

Furthermore, the present invention provides vaccines for treating and/or preventing a disease caused by neovascularization in the choroid, comprising at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof as an active ingredient. In the present invention, a vaccine refers to a composition which, when administered to an organism, can induce immune responses in vivo in that organism. In the present invention, immune responses induced in vivo refer to, in particular, induction of CTLs targeting cells expressing VEGFR-1. Since vascular endothelial cells involved in neovascularization in the choroid express VEGFR-1 on the cell surface, they may become targets of CTLs induced by administration of this vaccine. That is, administration of the vaccine of the present invention causes the peptides of the present invention to be presented at high density on the HLA antigens of the antigen-presenting cells, this induces CTLs which react specifically to the complex formed between the presented peptide and HLA antigen, and the power to attack vascular endothelial cells in the choroid is increased. Alternatively, antigen-presenting cells having peptides of the present invention on their cell surface are obtained by extracting dendritic cells from a patient and stimulating them with the peptides of the present invention. Returning the cells to the patient through administration causes CTL induction in the patient, and the power to attack vascular endothelial cells in the choroid can be increased.

The pharmaceutical compositions and vaccines of the present invention are effective against diseases caused by neovascularization in the choroid. There is no limitation on the target diseases of the pharmaceutical compositions and vaccines of the present invention, as long as they are diseases caused by choroid neovascularization. Preferably, the diseases include neovascular maculopathy that associate with diseases such as exudative age-related macular degeneration, myopic macular degeneration, angioid streaks, central exudative chorioretinopathy, various retinal pigment epitheliopathies, choroidal atrophy, choroideremia, and choroidal osteoma. A particularly preferred example is exudative age-related macular degeneration. The pharmaceutical compositions and vaccines of the present invention selectively attack vascular endothelial cells and thus have a low risk of rapid visual reduction and development of severe complications post-treatment, which are problems in conventional therapeutic methods. Therefore, the pharmaceutical compositions of the present invention can be applied not only to patients with severe symptoms but also to early-stage patients with relatively good vision. Since retinal damage is low in early-stage cases with relatively good vision, the visual prognosis post-treatment for advanced cases is expected to be much more favorable than in conventional treatment. Furthermore, pharmaceutical compositions and vaccines of the present inven-

tion have been confirmed to show effects in cases that do not respond to conventional therapeutic methods, and can be applied to such cases.

The present invention is based on the finding that neovascularization in the choroid is inhibited by administration of VEGFR-1 peptides. Therefore, the present invention provides pharmaceutical compositions for inhibiting neovascularization in the choroid, comprising at least one type each of a VEGFR-1 peptide and/or a polynucleotide encoding thereof. Furthermore, pharmaceutical compositions comprising VEGFR-1 peptides and/or a polynucleotide encoding thereof can be used as vaccines. Therefore, the present invention also provides vaccines for inhibiting neovascularization in the choroid, comprising at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof. The degree of inhibition is not particularly limited, and as long as neovascularization can be inhibited, even if the degree is slight, it is included in the meaning of inhibition.

The pharmaceutical compositions and vaccines of the present invention are not particularly limited so long as they contain at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof, and for example, they may comprise multiple types of VEGFR-1 peptides and/or any other substances, for example immune stimulators. Preferably, a peptide comprising an amino acid sequence derived from other protein and having an activity of inducing cytotoxic T cells can be comprised. More preferably, a peptide comprising an amino acid sequence derived from a VEGFR-2 protein and having an activity of inducing cytotoxic T cells (hereinafter referred to as "VEGFR-2 peptide"). The pharmaceutical compositions and vaccines of the present invention may contain, in addition to peptides, carriers, excipients, and such commonly used for pharmaceuticals when appropriate. For example, they may be used parenterally in the injectable form of sterile solutions or suspensions prepared with water or other pharmaceutically acceptable liquids. They may be formulated by appropriately combining them with pharmaceutically acceptable carriers or vehicles, more specifically, sterilized water or physiological saline solutions, vegetable oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binding agents, and such, and mixing them at a unit dosage form required by generally accepted pharmaceutical practice. The amount of active ingredient in these formulations is included to achieve appropriate doses within specified limit.

When the present invention is a vaccine, it may include an adjuvant so that cellular immunity is effectively established, and they may also include other active ingredients for neovascular maculopathy and such. They may also be made into particulate formulations. For adjuvants, those described in the document (Johnson A G., Clin. Microbiol. Rev., 7:277-289, 1994) or such are available. Other formulations may be liposome preparations, granular preparations produced by binding to micrometer-diameter beads, or lipid-bound preparations.

The amount of VEGFR-1 peptide contained in the pharmaceutical compositions and vaccines of the present invention is not particularly limited as long as it is a pharmaceutically effective amount. For example, an effective amount of each peptide may be 0.001 mg to 1000 mg, preferably 0.001 mg to 1000 mg, and more preferably 0.1 mg to 10 mg. Furthermore, if the pharmaceutical compositions and vaccines contain VEGFR-2 peptide, the combining ratio of the VEGFR-1 peptide to the VEGFR-2 peptide is not particularly limited, as long as pharmaceutically effective amounts of both peptides are contained. The amounts of VEGFR-1 peptide and VEGFR-2 peptide combined may be the same, or the amount of either one of the peptides combined may be greater than the other peptide. While VEGFR-2 is expressed on the surface of almost all vascular endothelial cells, VEGFR-1 is

expressed only on the surface of a specific portion of vascular endothelial cells; therefore, the amount of the VEGFR-2 peptide combined can be greater than that of the VEGFR-1 peptide.

In another embodiment of the present invention, the peptides of the present invention may also be administered in the form of a pharmaceutically acceptable salt. Preferable examples of the salts include salts with an alkali metal, salts with a metal, salts with an organic base, salts with an organic acid and salts with an inorganic acid.

The present invention also includes the use of VEGFR-1 peptide and/or a polynucleotide encoding thereof in manufacturing pharmaceutical compositions or vaccines for treating and/or preventing diseases caused by neovascularization in human choroid. Furthermore, the present invention includes the use of VEGFR-1 peptide and/or a polynucleotide encoding thereof in manufacturing pharmaceutical compositions or vaccines for inhibiting neovascularization in human choroid.

The present invention includes VEGFR-1 peptides and/or a polynucleotide encoding thereof to be administered to subjects for treating and/or preventing diseases caused by neovascularization in human choroid. In addition, the present invention includes VEGFR-2 peptides and/or a polynucleotide encoding thereof to be administered to subjects together with a VEGFR-1 peptide and/or a polynucleotide encoding thereof for treating and/or preventing diseases caused by neovascularization in human choroid. Furthermore, the present invention includes VEGFR-2 peptides to and/or a polynucleotide encoding thereof be administered to subjects together with a VEGFR-1 peptide and/or a polynucleotide encoding thereof for inhibiting neovascularization in human choroid. Additionally, the present invention includes VEGFR-1 peptides and/or a polynucleotide encoding thereof to be administered to subjects together with a VEGFR-2 peptide and/or a polynucleotide encoding thereof for inhibiting neovascularization in human choroid.

Kits for Treating or Preventing Neovascular Maculopathy and Kits for Inhibiting Neovascularization in the Choroid

The present invention provides kits for treating and/or preventing diseases caused by neovascularization in the choroid, comprising at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof. The present invention also provides kits for inhibiting neovascularization in the choroid, comprising at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof.

The VEGFR-1 peptide to be included in the kits of the present invention may be present individually alone, or they may exist in the form of formulations or vaccines by appropriately combining with pharmaceutically acceptable carriers or vehicles, or more specifically, sterilized water or physiological saline solutions, vegetable oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binding agents, and such. When they are produced into vaccines, an adjuvant may be included so that cellular immunity is effectively established, and other active ingredients for neovascular maculopathy and such may also be included. Preferably, VEGFR-2 peptide can be included. They may also be made into granular formulations. For adjuvants, those described in the document (Johnson A G., Clin. Microbiol. Rev., 7:277-289, 1994) or such are available. Other formulations may be liposome preparations, granular preparations produced by binding to micrometer-diameter beads, or lipid-bound preparations.

The kits of the present invention may further include pharmaceutically acceptable carriers or vehicles such as those described above so that one who prepares the pharmaceuticals can make appropriate adjustments.

Methods for Treating or Preventing Neovascular Maculopathy, and Methods for Inhibiting Neovascularization in the Choroid

The present invention further provides methods for treating and/or preventing diseases caused by neovascularization in the choroid, comprising the step of administering to a subject at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof. Furthermore, the present invention provides methods for inhibiting neovascularization in the choroid, comprising the step of administering to a subject at least a VEGFR-1 peptide and/or a polynucleotide encoding thereof.

VEGFR-1 peptide can be administered to subjects parenterally in the injectable form of sterile solutions or suspensions prepared with water or other pharmaceutically acceptable liquids. They may also be administered to subjects in the form of a formulation by appropriately combining with pharmaceutically acceptable carriers or vehicles, more specifically, sterilized water or physiological saline solutions, vegetable oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binding agents, and such, and mixing them at a unit dosage form required for generally accepted pharmaceutical practice. When administering VEGFR-1 peptide as vaccines, they may be administered together with an adjuvant so that cellular immunity is effectively established, and they may also be administered together with other active ingredients for neovascular maculopathy and such. For adjuvants, those described in the document (Johnson A G., Clin. Microbiol. Rev., 7:277-289, 1994) or such are available. VEGFR-2 peptide may also be administered together.

Those skilled in the art can suitably plan the method of administration, dose, and period of administration of VEGFR-1 according to the symptoms of patients (subjects) needing administration of the peptides of the present invention. The VEGFR-1 peptide can be administered to subjects as pharmaceutical compositions or vaccines of the present invention, or they may be administered to subjects as pharmaceutical compositions or vaccines containing each of the peptides individually. The VEGFR-1 peptide can be administered by either systemic administration or local administration. Examples of systemic administration include oral administration, intradermal administration, subcutaneous administration, and intravenous injection. Examples of local administration include administration to the vicinity of the choroid.

The dose of VEGFR-1 peptide may be, for example, 0.001 mg to 1000 mg, preferably 0.001 mg to 1000 mg, and more preferably 0.1 mg to 10 mg, but is not limited thereto. Furthermore, without limitation, the vaccines are preferably administered once in a few days or a few months, and more preferably once a week.

EXAMPLES

Hereinbelow, the present invention will be specifically described with reference to the Examples, but it is not to be construed as being limited thereto.

Example 1

Subjects

HLA-A0201-Case 1

As a subject, a 67-year old male patient with age-related macular degeneration who has been treated by photodynamic therapy and Avastin administration was selected. It is a case that did not go to remission by conventional therapeutic methods. Examination of the HLA-A locus confirmed that the subject carries HLA-A*0201.

HLA-A0201-Case 3

As a subject, a 76-year old male patient with age-related macular degeneration who has been treated by injection of a steroid (triamcinolone) below Tenon's capsule, photodynamic therapy, and Avastin administration was selected. It is a case that did not go to remission by conventional therapeutic methods. Examination of the HLA-A locus confirmed that the subject carries HLA-A*0201.

HLA-A2402-Case 1

As a subject, a 67-year old male patient with a age-related macular degeneration patient was selected. It is a case that did not go to remission by conventional therapeutic methods. Examination of the HLA-A locus confirmed that the subject carries HLA-A*2402.

Peptides

HLA-A*2402 restricted VEGFR1 peptide (VEGFR1-A24-1084; SYGVLLWEI; SEQ ID NO:4), HLA-A*2402 restricted VEGFR2 peptide (VEGFR2-A24-169; RFVPDGNRI; SEQ ID NO:8), HLA-A*0201 restricted VEGFR1 peptide (VEGFR1-A2-770; TLFWLLTL; SEQ ID NO: 2) and HLA-A*0201 restricted VEGFR2 peptide (VEGFR2-A2-773; VIAMFFWLL; SEQ ID NO: 12) of Good Manufacturing Practice (GMP) grade, HLA-A*2402-restricted HIV-Env protein-derived peptide (HIV-A24; RYL RDQQL; SEQ ID NO: 22) and HLA-A*0201-restricted HIV-Env protein-derived peptide (HIV-A2; SLYNTYATL; SEQ ID NO: 23) were synthesized and analyzed the quality by the American Peptide Company Inc. (Sunnyvale, Calif.).

Method of Administration

The GMP grade synthetic peptides, VEGFR-1 peptide (TLFWLLTL; SEQ ID NO: 2) and VEGFR-2 peptide (VIAMFFWLL; SEQ ID NO: 12), were obtained from the Human Genome Center, Institute of Medical Sciences, the University of Tokyo. One milligram each of the VEGFR-1 peptide and the VEGFR-2 peptide was mixed with 1 mL of incomplete Freund's adjuvant (MONTANIDE*ISA51VG, SEPPIC, France), and they were administered subcutaneously to the armpit of the patient. The administration was carried out once a week.

PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from patients (HLA-A*2402 or HLA-A*0201 positive) by Ficoll-Plaque (Pharmacia) solution.

IFN-Gamma ELISPOT Assay

Before the treatment and at the every end of treatment course, PBMCs were obtained and immediately frozen. For immune monitoring, all frozen PBMCs derived from each patient were thawed at the same time, and stimulated with 10 micro g/ml of the cognate peptide and 20 IU/mL of interleukin-2 (Chiron, Emeryville, Calif.) at 37 degrees C. with 5% CO₂ condition for two weeks. After the depletion of CD4⁺ cells by Dynal CD4 positive isolation kit (Invitrogen, Carlsbad, Calif.), cells were applied for interferon-gamma (IFN-gamma) enzyme-linked immunospot (ELISPOT) assay. IFN-gamma ELISPOT assay was performed according to manufacture's procedure (BD Biosciences, San Jose, Calif.). Briefly, HLA-A*2402-positive B-lymphoblast TISI cells (IHWG Cell and Gene Bank, Seattle, Wash.) or HLA-A*0201-positive B-lymphoblast T2 cells (ATCC, Tokyo, Japan) were incubated with 20 micro g/ml of the cognate peptide or HIV-Env peptide over night. After washing out the remaining peptide that not bind to HLA on the cells, respective peptide-pulsed cells (2×10⁴ cells/well) were used to stimulate prepared CD4⁻ cells (1×10⁴ cells/well) on 96-well plate (Millipore, Bedford, Mass.) at 37 degrees C. with 5% CO₂ condition over night. The plates were scanned and analyzed on an ImmunoSpot S4 Analyzer and ImmunoSpot

image analyzer software version 5.0 (Cellular Technology Ltd., Cleveland, Ohio). The number of the cognate peptide specific spots was calculated by subtracting the number of spots when stimulated with HIV-Env peptide from the number of spots when stimulated with the cognate peptide. All ELISPOT assays were performed triplicate wells. When the excess spots were detected, it is unable to calculate the accurate spot counts because of the clustering and those wells were defined to be saturated.

Flow Cytometric Analysis

To detect peptide specific T cell receptor, 5×10^5 of CD4⁺ cells prepared for ELISPOT assay were stained with phycoerythrin (PE)-conjugated HLA-A*2402/VEGFR1 dextramer or HLA-A*0201/VEGFR1 dextramer (DAKO Japan, Tokyo, Japan), fluorescein isothiocyanate (FITC)-conjugated anti-human CD8 mAb (RPA-T8, BD Biosciences, San Jose, Calif.) and allophycocyanina (APC)-conjugated anti-human CD3 mAb (UCHT1, BD Biosciences, San Jose, Calif.), then analyzed using FACSCanto II (BD Biosciences, San Jose, Calif.). HLA-A*2402/HIV-Env dextramer or HLA-A*0201/HIV-Env dextramer (DAKO Japan, Tokyo, Japan) were used as negative controls. Dead cells were excluded from the analysis based on the staining with 7-ADD (Sigma-Aldrich Japan, Tokyo, Japan).

Results

HLA-A0201-Case1

The progression stage of age-related macular degeneration was analyzed using optical coherence tomography, fluorescein fundus imaging, and fundus photography. Before starting administration of the VEGFR-1 peptide and the VEGFR-2 peptide, a large detachment of pigment epithelium was observed in the tomographic images of optical coherence tomography (FIG. 1A). Detachment of pigment epithelium was clearly observed also in the fundus photograph (FIG. 1B). Furthermore, a large amount of leakage was observed in the image of fluorescein fundus photography (FIG. 1D).

Five months after starting administration of the VEGFR-1 peptide and the VEGFR-2 peptide, a significant decrease in the detachment of pigment epithelium was observed in the tomographic images of optical coherence tomography (FIG. 1D). Amelioration of the detachment of pigment epithelium was also observed with fundus photography (FIG. 1E). Furthermore, reduced leakage was confirmed in the fluorescein fundus photograph (FIG. 1F). It was also reported that subjective symptoms such as distortion were greatly ameliorated. The vision of the right eye was slightly improved ($R_v = (0.9) \rightarrow R_v = (1.2)$). These results confirmed that administration of the VEGFR-1 peptide and the VEGFR-2 peptide yields amelioration effects for age-related macular degeneration. Problems suggestive of safety issue did not arise.

HLA-A0201-Case3

Before starting administration of the VEGFR-1 peptide and the VEGFR-2 peptide, rupture of the retina due to leakage from the neovessels and edema in the retina were observed in the tomographic images of optical coherence tomography (FIG. 2A). One month after starting administration of the VEGFR-1 peptide and the VEGFR-2 peptide, edema of the retina was clearly found to be reduced compared to before the administration was started (FIG. 2B). Furthermore, an apparently fibrosed and hypoactive neovascular membrane was observed (FIG. 2B). Furthermore, it was reported that subjective symptoms such as distortion were significantly ameliorated. These results confirmed that administration of the VEGFR-1 peptide and the VEGFR-2 peptide yields amelioration effects for the symptoms of age-related macular degeneration in this case as well. Problems suggestive of safety issue did not arise.

HLA-A2402-Case1

Before starting administration of the VEGFR-1 peptide and the VEGFR-2 peptide, clear subretinal hemorrhages were observed in the ocular fundus photographs (FIG. 3, upper left panel). Three month after starting administration, the subretinal hemorrhages were obviously relieved compared with before administration (FIG. 3 upper center and right panels). Furthermore, anatomy of macular region have no effect (FIG. 3, lower panel) and the vision was improved.

Monitoring Analysis

IFN-gamma ELISPOT assay and/or Flow cytometric analysis were performed as monitoring of patient treated.

TABLE 1

Summary of monitoring analysis						
Dose	Case	Treatment course	CTL response			Multimer analysis
			R1	R2	CMV	CD8 ⁺ R1dextramer ⁺ / CD3 ⁺ CD4 ⁻ (%)
1 mg	HLA-A0201-Case 1	pre-treatment	+++	-	+++	NT
		post-1course	+++	-	+++	NT
		post-2course	+++	-	+++	NT
		post-3course	+++	-	+++	NT
		post-4course	+++	-	+++	NT
	HLA-A0201-Case 3	pre-treatment	+	+	+++	0.01
		post-1course	+++	-	+++	0.05
		post-2course	NT	NT	NT	NT
		post-3course	+++	-	+++	0.69
		post-4course	+++	-	+++	0.04
HLA-A2402-Case 1	pre-treatment	-	-	+++	NT	
	post-1course	-	-	+	NT	
	post-2course	+++	-	-	NT	
	post-3course	++	+	++	NT	
	post-4course	+	-	-	NT	
	post-5course	+++	++	+++	NT	
		post-6course	++	-	-	NT

NT: not tested

HLA-A0201-Case1

Significantly higher number of spots were observed when stimulated with VEGFR1-A2-770 peptide-pulsed T2 cells compared with that stimulated with HIV-Env peptide-pulsed T2 cells in IFN-gamma ELISPOT assay, especially after treatment courses (Table 1 and FIG. 4). On the other hand, no specific IFN-gamma production was observed by stimulation with VEGFR2-A2-773 peptide (Table 1 and FIG. 5), despite administration of both VEGFR1-A2-770 peptide and VEGFR2-A2-773 peptide shown obvious efficacy in the patient. As a result, it indicated that VEGFR1-A2-770 peptide function to improve the case alone.

HLA-A0201-Case3

Significantly higher number of spots were observed when stimulated with VEGFR1-A2-770 peptide-pulsed T2 cells compared with that stimulated with HIV-Env peptide-pulsed T2 cells in IFN-gamma ELISPOT assay (Table 1 and FIG. 6). Consistently, significant population of HLA-A*0201/VEGFR1-A2-770 dextramer+CD8⁺ cells were detected by flow cytometric analysis (FIG. 6 lower panels). On the other hand, no specific IFN-gamma production was observed by stimulation with VEGFR2-A2-773 peptide (Table 1 and FIG. 7), despite administration of both VEGFR1-A2-770 peptide and VEGFR2-A2-773 peptide shown obvious efficacy in the patient. As a result, it indicated that VEGFR1-A2-770 peptide function to improve the case alone.

HLA-A2402-Case1

Significantly higher number of spots were observed when stimulated with VEGFR1-A24-1084 peptide-pulsed TISI cells compared with that stimulated with HIV-Env peptide-

pulsed TISI cells in IFN-gamma ELISPOT assay, especially after treatment courses (Table 1 and FIG. 8). On the other hand, no specific IFN-gamma production was observed by stimulation with VEGFR2-A24-169 peptide (Table 1 and FIG. 9), despite administration of both VEGFR1-A24-1084 peptide and VEGFR2-A24-169 peptide shown obvious efficacy in the patient. As a result, it indicated that VEGFR1-A24-1084 peptide function to improve the case alone.

Change in Vision After Treatment

The visions of treatment groups were improved with significant difference ($p=0.015$) (FIG. 10).

INDUSTRIAL APPLICABILITY

The present invention provides pharmaceutical compositions/vaccines for treatment and/or prevention of diseases caused by neovascularization in the choroid (neovascular maculopathy). Conventionally, laser therapy, photodynamic therapy, operative therapy, drug therapy, and such have been performed as therapeutic methods for neovascular maculopa-

thy. However, laser therapy could reduce central vision. There are examples of rapid visual reduction following photodynamic therapy in cases with good vision. In operative therapy, there is a risk of postoperative complications associated with surgical invasion. In drug therapy, there is a risk of serious complications such as endophthalmitis and retinal detachment due to intraocular injection. That is, conventional therapies have a high risk of visual reduction due to treatment-associated adverse effects and complications. Therefore, it was difficult to treat early-stage cases with relatively good vision. Since safety problems did not arise in the administered cases, one can expect the pharmaceutical compositions/vaccines of the present invention to provide low-risk and highly safe therapeutic agents and therapeutic methods for neovascular maculopathy. Furthermore, since they are shown to be effective for cases that do not respond to conventional therapeutic methods, it can be expected that they will provide therapeutic agents and therapeutic methods for cases for which conventional therapeutic methods have not been effective.

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<210> SEQ ID NO 19
<211> LENGTH: 1338
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 19

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          20          25          30
Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
          35          40          45
Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
          50          55          60
Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
          65          70          75          80
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
          85          90          95
Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
          100         105         110
Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
          115         120         125
Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
          130         135         140
Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
          145         150         155         160
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
          165         170         175
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
          180         185         190
Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
          195         200         205
Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
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Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
          225         230         235         240
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
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Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
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Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
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Ser	Val	Asn	Thr	Ser	Val	His	Ile	Tyr	Asp	Lys	Ala	Phe	Ile	Thr	Val
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Lys	His	Arg	Lys	Gln	Gln	Val	Leu	Glu	Thr	Val	Ala	Gly	Lys	Arg	Ser
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Tyr	Arg	Leu	Ser	Met	Lys	Val	Lys	Ala	Phe	Pro	Ser	Pro	Glu	Val	Val
		355					360					365			
Trp	Leu	Lys	Asp	Gly	Leu	Pro	Ala	Thr	Glu	Lys	Ser	Ala	Arg	Tyr	Leu
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Thr	Arg	Gly	Tyr	Ser	Leu	Ile	Ile	Lys	Asp	Val	Thr	Glu	Glu	Asp	Ala
385					390					395					400
Gly	Asn	Tyr	Thr	Ile	Leu	Leu	Ser	Ile	Lys	Gln	Ser	Asn	Val	Phe	Lys
				405					410					415	
Asn	Leu	Thr	Ala	Thr	Leu	Ile	Val	Asn	Val	Lys	Pro	Gln	Ile	Tyr	Glu
			420					425					430		
Lys	Ala	Val	Ser	Ser	Phe	Pro	Asp	Pro	Ala	Leu	Tyr	Pro	Leu	Gly	Ser
		435					440					445			
Arg	Gln	Ile	Leu	Thr	Cys	Thr	Ala	Tyr	Gly	Ile	Pro	Gln	Pro	Thr	Ile
	450					455					460				
Lys	Trp	Phe	Trp	His	Pro	Cys	Asn	His	Asn	His	Ser	Glu	Ala	Arg	Cys
465					470					475					480
Asp	Phe	Cys	Ser	Asn	Asn	Glu	Glu	Ser	Phe	Ile	Leu	Asp	Ala	Asp	Ser
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Asn	Met	Gly	Asn	Arg	Ile	Glu	Ser	Ile	Thr	Gln	Arg	Met	Ala	Ile	Ile
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Glu	Gly	Lys	Asn	Lys	Met	Ala	Ser	Thr	Leu	Val	Val	Ala	Asp	Ser	Arg
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Ile	Ser	Gly	Ile	Tyr	Ile	Cys	Ile	Ala	Ser	Asn	Lys	Val	Gly	Thr	Val
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Gly	Arg	Asn	Ile	Ser	Phe	Tyr	Ile	Thr	Asp	Val	Pro	Asn	Gly	Phe	His
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Val	Asn	Leu	Glu	Lys	Met	Pro	Thr	Glu	Gly	Glu	Asp	Leu	Lys	Leu	Ser
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Cys	Thr	Val	Asn	Lys	Phe	Leu	Tyr	Arg	Asp	Val	Thr	Trp	Ile	Leu	Leu
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Arg	Thr	Val	Asn	Asn	Arg	Thr	Met	His	Tyr	Ser	Ile	Ser	Lys	Gln	Lys
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Val	Tyr	Thr	Gly	Glu	Glu	Ile	Leu	Gln	Lys	Lys	Glu	Ile	Thr	Ile	Arg
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Asp	Gln	Glu	Ala	Pro	Tyr	Leu	Leu	Arg	Asn	Leu	Ser	Asp	His	Thr	Val
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Ala	Ile	Ser	Ser	Ser	Thr	Thr	Leu	Asp	Cys	His	Ala	Asn	Gly	Val	Pro
		675					680					685			
Glu	Pro	Gln	Ile	Thr	Trp	Phe	Lys	Asn	Asn	His	Lys	Ile	Gln	Gln	Glu
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Pro	Gly	Ile	Ile	Leu	Gly	Pro	Gly	Ser	Ser	Thr	Leu	Phe	Ile	Glu	Arg	705	710	715	720
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Asp	Lys	Ser	Asn	Leu	Glu	Leu	Ile	Thr	Leu	Thr	Cys	Thr	Cys	Val	Ala	755	760	765	
Ala	Thr	Leu	Phe	Trp	Leu	Leu	Leu	Thr	Leu	Phe	Ile	Arg	Lys	Met	Lys	770	775	780	
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Pro	Asp	Glu	Val	Pro	Leu	Asp	Glu	Gln	Cys	Glu	Arg	Leu	Pro	Tyr	Asp	805	810	815	
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Ile	Leu	Thr	His	Ile	Gly	His	His	Leu	Asn	Val	Val	Asn	Leu	Leu	Gly	885	890	895	
Ala	Cys	Thr	Lys	Gln	Gly	Gly	Pro	Leu	Met	Val	Ile	Val	Glu	Tyr	Cys	900	905	910	
Lys	Tyr	Gly	Asn	Leu	Ser	Asn	Tyr	Leu	Lys	Ser	Lys	Arg	Asp	Leu	Phe	915	920	925	
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Cys	Asp	Phe	Gly	Leu	Ala	Arg	Asp	Ile	Tyr	Lys	Asn	Pro	Asp	Tyr	1040	1045	1050		
Val	Arg	Lys	Gly	Asp	Thr	Arg	Leu	Pro	Leu	Lys	Trp	Met	Ala	Pro	1055	1060	1065		
Glu	Ser	Ile	Phe	Asp	Lys	Ile	Tyr	Ser	Thr	Lys	Ser	Asp	Val	Trp	1070	1075	1080		
Ser	Tyr	Gly	Val	Leu	Leu	Trp	Glu	Ile	Phe	Ser	Leu	Gly	Gly	Ser	1085	1090	1095		
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1145						1150					1155			
Gln	Ala	Asn	Val	Gln	Gln	Asp	Gly	Lys	Asp	Tyr	Ile	Pro	Ile	Asn
1160						1165					1170			
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1175						1180					1185			
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Ser	Lys	Glu	Ser	Gly	Leu	Ser	Asp	Val	Ser	Arg	Pro	Ser	Phe	Cys
1280						1285					1290			
His	Ser	Ser	Cys	Gly	His	Val	Ser	Glu	Gly	Lys	Arg	Arg	Phe	Thr
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Tyr	Asp	His	Ala	Glu	Leu	Glu	Arg	Lys	Ile	Ala	Cys	Cys	Ser	Pro
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<210> SEQ ID NO 20

<211> LENGTH: 6055

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

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<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

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The invention claimed is:

1. A method for treating a disease caused by neovascularization in human choroid, comprising the step of administering to a subject a pharmaceutical composition comprising, as active ingredients, at least one peptide selected from the group consisting of:

- (i) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4;
- (ii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine;
- (iii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;

- (iv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;

- (v) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan;

- (vi) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine; and

- (vii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine, and

at least one peptide selected from the group consisting of:

- (viii) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 12; and

- (ix) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine;

- (x) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;

- (xi) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;

- (xii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan;

- (xiii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine; and

- (xiv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine.

2. The method of claim 1, wherein the disease caused by neovascularization in the choroid is selected from exudative age-related macular degeneration, myopic macular degeneration, angioid streaks, central exudative chorioretinopathy, various retinal pigment epitheliopathy, choroidal atrophy, choroideremia, and choroidal osteoma.

3. The method of claim 1, which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24.

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4. A method of treating a disease caused by neovascularization in human choroid, comprising the step of administering to a subject a vaccine comprising, as active ingredients, at least one peptide selected from the group consisting of:

- (i) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4;

- (ii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine;

- (iii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;

- (iv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;

- (v) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan;

- (vi) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine; and

- (vii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine,

and at least one peptide selected from the group consisting of:

- (viii) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 12; and

- (ix) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine;

- (x) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;

- (xi) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;

- (xii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan;

- (xiii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine; and

- (xiv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine.

5. The method of claim 4, wherein the disease caused by neovascularization in the choroid is selected from exudative age-related macular degeneration, myopic macular degeneration, angioid streaks, central exudative chorioretinopathy, various retinal pigment epitheliopathy, choroidal atrophy, choroideremia, and choroidal osteoma.

6. The method of claim 4, which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24.

7. A method for inhibiting neovascularization in human choroid, comprising the step of administering to a subject a

pharmaceutical composition comprising, as active ingredients, at least one peptide selected from the group consisting of:

- (i) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4;
 - (ii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine;
 - (iii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;
 - (iv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;
 - (v) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan;
 - (vi) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine; and
 - (vii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine, and
- at least one peptide selected from the group consisting of:
- (viii) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 12; and
 - (ix) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine;
 - (x) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;
 - (xi) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;
 - (xii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan;
 - (xiii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine; and
 - (xiv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine.

8. The method of claim **7**, which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24.

9. A method for inhibiting neovascularization in human choroid, comprising the step of administering to a subject a vaccine comprising, as active ingredients, at least one peptide selected from the group consisting of:

- (i) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4,
- (ii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine;

- (iii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;
 - (iv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 2 is modified to methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2 is modified to valine;
 - (v) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan;
 - (vi) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine; and
 - (vii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 4 is modified to phenylalanine, leucine, tryptophan or methionine, and
- at least one peptide selected from the group consisting of:
- (viii) a peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 12; and
 - (ix) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine;
 - (x) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;
 - (xi) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 12 is modified to leucine or methionine and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 12 is modified to valine;
 - (xii) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan;
 - (xiii) a peptide in which the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine; and
 - (xiv) a peptide in which the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 8 is modified to tyrosine, methionine or tryptophan and the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 8 is modified to phenylalanine, leucine, tryptophan or methionine.

10. The method of claim **9**, which is administered to a subject whose HLA antigen is HLA-A02 or HLA-A24.

11. The method of claim **1**, wherein the amount of the peptide selected from the group consisting of (i) to (vii) is 0.1 mg to 10 mg.

12. The method of claim **11**, wherein the amount of the peptide selected from the group consisting of (viii) to (xiv) is the same amount as the peptide selected from the group consisting of (i) to (vii).

13. The method of claim **4**, wherein the amount of the peptide selected from the group consisting of (i) to (vii) is 0.1 mg to 10 mg.

14. The method of claim **13**, wherein the amount of the peptide selected from the group consisting of (viii) to (xiv) is the same amount as the peptide selected from the group consisting of (i) to (vii).

15. The method of claim **4**, wherein the vaccine is administered together with an adjuvant.

16. The method of claim **15**, wherein the adjuvant is incomplete Freund's adjuvant.

17. The method of claim 7, wherein the amount of the peptide selected from the group consisting of (i) to (vii) is 0.1 mg to 10 mg.

18. The method of claim 17, wherein the amount of the peptide selected from the group consisting of (viii) to (xiv) is 5 the same amount as the peptide selected from the group consisting of (i) to (vii).

19. The method of claim 9, wherein the amount of the peptide selected from the group consisting of (i) to (vii) is 0.1 mg to 10 mg. 10

20. The method of claim 19, wherein the amount of the peptide selected from the group consisting of (viii) to (xiv) is the same amount as the peptide selected from the group consisting of (i) to (vii).

21. The method of claim 9, wherein the vaccine is administered together with an adjuvant. 15

22. The method of claim 21, wherein the adjuvant is incomplete Freund's adjuvant.

* * * * *